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GENETICAL CONTROL OF STABILITY IN DEVELOPMENT

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I. CANALISATION AND STABILISATION

CELLS and tissues may follow a variety of paths during the normal course of development and differentiation, even though they are the same or closely similar in genotype. These paths are, however, far from haphazard: as Waddington (1942) has emphasised, they are limited in number and sharply distinct from one another. Intermediates are absent, so that development may be said to be canalised.

The choice of path from among the possibilities open to the cell may be determined immediately by a particular nuclear relation. Thus in diœcious animals the decision between male and female development commonly depends on segregation of the X-Y difference, on whether the zygote is carrying XX or XY chromosomes. This genetical difference is sometimes large, involving whole chromosomes or even more than one chromosome; but in other cases it seems to be much more simple, consisting perhaps in little more than a single gene, if we may judge by the lack of cytological differentiation in mosquitoes (Gilchrist and Haldane, 1947), by the ease of transference of the switching function in fishes (Winge and Ditlevsen, 1947; Gordon, 1947), and by the ability of the YY type to survive and function in axolotls (Humphrey, 1942) and asparagus (Rick and Hanna, 1943). In a few species (see White, 1945), the course of sexual development is switched by external causes. Environmental switches of this kind are rare, most probably because they have been replaced by the more certain and more precise switching that genes can give (Waddington loc. cit.).

These are cases where the differences are seen in the development of complete individuals. The same canalisation is, however, observable in the differentiation of tissues within a single soma, and here there is no ground for regarding the switch into one channel or another as being due to any difference, even the smallest, in genotype. Rather the critical difference is to be sought in the cytoplasm which is changing serially under the control of a constant nuclear genotype, the earliest difference being in the cytoplasm of the egg cell, where it was laid down by the relation of the cell to maternal tissue, or to

such external forces as gravity (Mather 1948*a* and *b*). But whatever the critical difference, whether genetic or environmental as we see it in sex determination, or cytoplasmic as we see it in differentiation, the principle is the same. One of a limited number of courses will be followed in development, depending on a difference often so simple as to seem almost trivial. Indeed the difference may be demonstrably trivial under other circumstances, for the changeover of sex determination in fishes from one gene to another shows us that a genetic element whose segregation is critical in one strain may be without influence on sexual development in another strain of the same species. Nor do external forces have such an effect at later stages in development as they appear to have in determining the cytoplasmic arrangement of the egg in some species, though they can hardly have ceased to operate.

Thus, differences which are critical at one time or under one set of circumstances are ineffective on other occasions. This obviously suggests that the genotype is adjusted to confer on them their power of switching development, to build on an initial small difference in the sense that, given the initial difference, the genotype will determine a divergence. Experiment has confirmed that such is the case. Alteration of the genotype can lead to a digression in the course of the channel into which development is switched. Genotypes can be produced in which, for example, the X-Y difference no longer determines operative males versus females, or in which the normal course of tissue differentiation breaks down. The course of development, with its various alternatives no matter how they are switched, is inherent in the genotype : the outcome of development is successful, and the intermediates between the various channels are absent, because natural selection has adjusted the genotype to give just this result.

This regulation of canalisation cannot stop at building on the initial and critical differences : once the initial difference between the development of male and female or between tissues of the same soma has been laid down, later disturbances of kinds normally encountered appear unable to divert the development from its destined course. It can hardly be doubted, however, that disturbances must occur. The environment can never be without its action, which may well differ in different parts of a soma as well as vary from one individual to another. Furthermore, there must be chance upsets in the cells themselves, failures, for example, of the cytoplasm to partition equally in both bulk and content at cell division where it should be equal, or to achieve a precise inequality where such inequality is normal. Such disturbances must tend to be accumulative, yet they appear to have no serious effect on the course of development. It would thus appear that the genotype which can be adjusted to offer a series of alternative channels can also be adjusted to keep

these channels within narrow bounds ; that it can be adjusted not only to build up, where advantageous, the effects of initial differences within the cell, but also to suppress them where they would be harmful.

The manifestation of mutant genes affords some evidence of this stabilising power of the genotype (Waddington l.c.). The expression of, for example, the gene " eyeless " in *Drosophila* is much more variable both between individuals and between the two sides of a single individual than is that of its normal allelomorph. The genotype appears to have been adjusted by natural selection to give a relatively uniform development of eye size in the normal fly, but the stability vanishes once the course of development is changed by the introduction of the mutant gene. The old balance is no longer effective and a new adjustment of the genotype would be necessary to achieve a corresponding stability in the new circumstances of development. Such lower variation between normal individuals will in large part be due to reduction in expression of the differences which must generally exist between their genotypes ; but the lesser differences between the two eyes of the same normal fly must be ascribed to a better regulation of the individual's own development, for there is no ground for regarding the genotype as other than effectively uniform within a fly.

The study of asymmetry affords therefore a means of investigating the genotypic stabilisation of developmental processes. Eye size is not, however, a convenient character to study metrically, because irregularity in shape can make the area difficult to measure directly and the high number of facets makes their counting troublesome. The number of sternopleural chaetæ in *Drosophila melanogaster* affords a much more convenient character. These chaetæ, lying between the fore and mid legs, are readily counted ; they normally number between 8 and 12 on each side ; they commonly show a measure of asymmetry ; they are known to be subject to heritable variation ; and their number is not so greatly upset by external conditions, such as crowding in the culture bottle, as are features like overall bodily dimensions. The present study has therefore been made on this character.

2. INBREEDING EXPERIMENTS

(a) Asymmetry as a reflection of instability

If the developmental paths are intrinsically the same on the two sides of the individual (implying, of course, not only that the genotype is the same, but that the seriation of cytoplasmic changes in differentiation is the same, and that there is no consistent difference in the action of external agencies) the expression of the character will be symmetrical, barring local upsets. Any asymmetry which may appear will be an expression of such local disturbances, whether arising from differences of environment or upsets of cell development ; but since

such disturbances (if they are fairly and wholly to be regarded as chance disturbances and not as, at least up to a point, regular features of development) should have equal chances of affecting each side, the average expression of the character should be symmetrical, even though any given individual may differ bilaterally. In other words, if we plot the frequency distribution of the chance difference between the sides, excess on one being arbitrarily taken as positive and on the other as negative, it should be symmetrical about a mean of zero within the limits of sampling variation. Where all the individuals contributing to the distribution are of the same genotype, the spread of the distribution, measured by its variance, will depend on the frequency and magnitude of the local upsets, and also on the capacity of that genotype to stabilise development in spite of upset. Thus given comparable circumstances the capacity of two genotypes for coping with upset may be compared through the variances of their distributions of differences between the two sides. Where the frequency distribution is obtained from individuals whose genotypes are not uniform, the variance may also reflect genetical differences, but it may still be taken as measuring the stabilising power of some average genotype in the sense that difference between the variances given by two populations raised under similar conditions would indicate a disparity in the ability of their overall genotypes to cope with disturbances in development.

Even where the average expressions of the character on the two sides were not exactly equal, so that there was a slight bias in favour of one of them or in other words a slight consistent differentiation between the sides in development, the variance of the distribution of differences between the sides would still, when corrected for the departure of the mean from zero, be a measure of stabilising power of the genotype. But if the averages of the sides were very different, as is the case with the expression of polydactyly in poultry (Fisher, 1935), the variance might well become a complex and unreliable measure of stabilising power, because developmental processes on the two sides must then be, for one reason or another, intrinsically different and so might react differently to upsets of similar cause.

Our first step must therefore be to check on bias between the sides. Given an absence or near absence of bias, we can take the variance of the bilateral difference as a measure of stabilising capacity, provided too that we are satisfied that different genotypes are compared over similar ranges of circumstances.

With these considerations in mind the experiments were begun using two long inbred stocks, Oregon (O) and Samarkand (S). Cultures of the two stocks, their reciprocal F_1s, and F_2s from these F_1s were raised on a number of occasions, a single pair being used as the parents of each culture and up to four cultures of a kind being produced on any one occasion. Though genetical variation is known

to exist in the O stock, and probably exists in S as well, its amount is so small that the stocks may be regarded as homogenic without serious error. The F_1s may therefore be taken as homogenic apart from differences between the reciprocals in the sex chromosomes of their males. The F_2s cannot of course be homogenic, but again apart from the sex chromosome differences of the two reciprocals, the various cultures will be genotypically comparable.

The numbers of sternopleural chaetæ were counted on each side of 20 males and 20 females in each of 25 cultures of the parent stocks (1000 flies from each stock), in each of 25 cultures from the two reciprocal F_1s (1000 flies from each reciprocal) and in each of 20 cultures from the two reciprocal F_2s (800 flies from each reciprocal). The number of chaeta on the right side (R) was subtracted from that on the left side (L) for each fly, a positive value thus indicating excess on the left and a negative value excess on the right.

The mean L−R difference should be zero if no bias of development is present. The average of L−R is given for the two series of parents, F_1s and F_2s in table 1, together with the average gross numbers of chaetæ, *i.e.* the average of L+R.

TABLE 1

Average values of L−R and L+R in parents, F_1s and F_2s

Generation	Bias (L−R)		Gross numbers of chaetæ (L+R)	
	Females	Males	Females	Males
Parents O	0·05	0·12	18·78	18·67
Parents S	0·08	0·16	19·76	19·62
F_1s O×S	0·05	0·16	19·44	18·93
F_1s S×O	0·03	0·08	19·35	18·90
F_2s O×S	−0·01	0·03	18·95	18·84
F_2s S×O	−0·11	0·02	19·24	18·89

The excess of L over R is significant in O, S and the two F_1s, but not in the F_2s where indeed R exceeds L on the average in females. It thus appears that there may be a bias in flies of a particular kind, but that this varies with the genotype and is in any case small. Taking flies of later generations (described below) also into account, the average bias was found to be 0·0303 of a chaeta over 8800 females, and 0·0926 over 8800 males, or 0·0615 over 17,600 flies of both sexes. Even in males this is only about 1 per cent. of the average number of chaetæ on a single side or 0·5 per cent. of the average number for the two sides taken together.

It is of interest to notice that the sex difference is consistent, not only in the generations of table 1, but also in the later generations.

The mean value of L—R in males exceeds that in females by 0·0623 over the whole 17,600 flies. For some reason males tend to a greater extent than females to produce slightly more chaetæ on L than on R. The higher bias of males is not the consequence of a higher gross number of chaetæ, for this is actually smaller than in females (table 1).

The average value of L—R also varies significantly between cultures of parents, F_1s and F_2s, and the variation is greater between cultures raised on different occasions than between those raised at the same time. Here again, however, the contribution of differences between cultures is small, being less than 2 per cent. of the variation within a single culture, even when measured between cultures raised on different occasions. Variation between cultures raised on the same occasion is barely significant ($P = 0{\cdot}049$). There is no good evidence of differences between the two parents, or between the reciprocals in F_1 and F_2, in the bias they show. Nor is there evidence of interaction in effect of generations, sex and culture.

The bilateral bias thus varies with sex, genotype and culture, but is always small. It can hardly be taken as indicating differences between the inherent developmental paths of the two sides sufficiently large to invalidate our use of the frequency distribution of L—R as a basis for comparing the stabilising capacities of the genotypes. The variances of the distributions of L—R differences given by the various cultures have, however, been corrected for the bias in nearly all later calculations in this Section (those of $2(d)$ being the exception), *i.e.* the variance of the distribution of L—R in each culture has been taken round the mean of L—R and not round zero.

(*b*) *Oregon, Samarkand and their F_1s and F_2s*

The variances of L—R are set out for the parents, F_1s and F_2s in table 2, males and females being given separately. Since there were 25 cultures of each parent and F_1, with 20 flies of each sex counted, each of these sex variances is based on $25 \times 19 = 475$ degrees of freedom (N). The pooled variance (V) of each parent and each reciprocal F_1 will thus be based on $N = 950$ degrees of freedom, and V for both reciprocal F_1s taken together on $N = 1900$. With 20 cultures of each F_2 V for the separate sexes will be based on $N = 380$, the pooled V for each of the F_2s on $N = 760$, and V for both F_2s together on $N = 1520$.

In the cases of parents and F_1s, estimates of V are available from 25 cultures, and from 20 in the F_2s. These 25 or 20 estimates may be tested for agreement with others by the method described by Stevens in the appendix to a paper by Fabergé (1936). In effect, this method compares the sum of squares of the 25 (or 20) estimates of V calculated round the overall mean estimate ($\bar{V}$), with the expected variance of V, found as $2\bar{V}^2$, to give a χ^2 for $N = 24$ (or 19). In no case was there any indication of heterogeneity of the estimates

of V from the individual cultures. Indeed summing the χ^2s over sexes and generations we find a value of 212·45 for N = 268 (=8×24+4×19). The variation between cultures is thus sub-normal if anything. The differences in conditions from one culture bottle to another have no detectable effect on the variation in asymmetry, even though they have a small effect on the bias. This finding is important in two ways. It tells us that conditions are sufficiently alike from one bottle to another to permit simple comparisons of the properties of genotypes grown in different cultures; and it suggests that the environmental component in asymmetry is not large, so that such asymmetry as is seen very likely springs in a large measure, not from local differences in the environment, but from chance cellular upsets in development.

Given that simple comparisons between genotypes raised in different cultures are legitimate, we may consider the implications of the figures in table 2. The most striking feature is provided by

TABLE 2

Variances of L—R (V) in parents, F_1s and F_2s

V in	Parents		F_1		F_2	
	O	S	O×S	S×O	O×S	S×O
Females . . .	2·0976	2·3854	1·8305	1·7751	1·4891	1·7108
Males . . .	2·0916	2·3116	1·8151	1·9247	1·7967	1·7405
Sexes pooled . .	2·0946	2·3485	1·8228	1·8499	1·6429	1·7257
	2·2216		1·8363		1·6843	

the differences between the generations. The Vs of the parent lines O and S can be compared by the method of Fisher (1950, p. 227). Their difference is not quite significant, having P just below 10 per cent. The pooled value of 1·8363 for the two F_1s taken together falls short of V for the lower parent (O) with a probability of between 3 and 4 per cent. The pooled value for both F_2s falls short of the F_1 V with a probability of between 7 and 8 per cent. Though extremely suggestive none of these comparisons is unambiguously significant in itself; but if we compare parents, F_1s and F_2s in a single test we find $\chi^2 = 36{\cdot}23$ for N = 2, even where the two parents are pooled. There can thus be no doubt that the different generations differ in their Vs, and there is reasonable presumptive evidence that O and S also differ in this respect. Evidently different genotypes give different degrees of stability in development. The further implications of these comparisons will be discussed in a later section.

It will be seen from table 2 that males and females are alike in V in the two parents, O being lower than S. The sexes do not appear to agree so well in the F_1 of S×O and the F_2 of O×S, though agreement in F_1(O×S) and F_2(S×O) is as good as in the parents. This suggests that the X chromosome of O carries one or more dominant genes for lower variance, or greater stability to put it in developmental terms. The action of such a gene or genes would result (i) in the males of F_1(S×O) having a higher V than either the males of the reciprocal F_1 or the females of both reciprocals, these females and the O×S males being alike in V ; and (ii) in the females of F_2(O×S) having a lower V than the females of F_2(S×O) or both sets of F_2 males, these F_2(S×O) females and both sets of males being alike in V.

Qualitatively these expectations are fully realised by the data, but when statistical tests are applied it is found that though higher than both sets of F_1 females and F_1(O×S) males, the F_1(S×O) males do not exceed them significantly in V ($\chi^2_{[1]} = 0{\cdot}73$) ; and equally that although lower than both sets of F_2 males and the F_2(S×O) females, the F_2(O×S) females fall short of them in V to an extent which is barely significant even when it is taken into account that the deviation is in the predicted direction ($\chi^2_{[1]} = 3{\cdot}40$). Thus, although suggesting, perhaps strongly, that the X chromosome of O carries a dominant gene or genes increasing the stability of development, the data cannot be taken as conclusively demonstrating this point.

Be the position in regard to sex linkage as it may, there can be no doubt that the variances of parents, F_1 and F_2 differ, so that the parent lines O and S must differ in genes affecting the stability of development. These genes should be segregating in the F_2s, but none of the tests so far described could reveal such segregation, for the F_2s have had to be considered as wholes, and, though not generally uniform like parents and F_1s, all the cultures of F_2 must have comparable ranges at genotypes. Later generations from the crosses, however, make possible tests of segregation, as we shall now see.

(c) *Later generations from the crosses*

Breeding was continued from the F_2s in two experiments made at different times. These experiments followed the same essential pattern : generations up to F_{10} were raised, all matings being of single pairs throughout. Each experiment traced back to four F_1 cultures, two of each reciprocal cross, made simultaneously. Two F_2s were raised from each F_1, *i.e.* four F_2s from each reciprocal, or eight in all. Two F_3s were next raised from each F_2 culture, making sixteen in all. In the first experiment this doubling process was continued for one further generation to give thirty-two F_4s, a pair from each F_3. Only one of each twin pair of F_4 cultures was, however, used to give parents for F_5, two F_5 cultures being set up from each of the sixteen F_4s so taken. There were thus thirty-two F_5s and the doubling

process had stopped. Sixteen F_5 cultures, one from each twin pair, were similarly used to give parents for F_6, two F_6s being made from each of these F_5s, and the process was repeated to F_{10}. The flies were taken as parents of the next generation without conscious selection: the first two females and the first two males to be counted were used. Nor was any conscious selection exercised in taking one of a pair of cultures to give parents of the next generation. As already stated, all matings were of a single pair, and the members of a pair were always full sibs. The matings being biparental, the generations were not F_3, F_4, etc., in the strict mendelian sense (see Mather, 1949), but this notation will be retained for convenience.

The second experiment was like the first in structure, but the doubling process stopped a generation earlier so that there were only sixteen F_4s, two from each of eight of the F_3s. In both experiments the reciprocal crosses contributed equally to the cultures of the various generations, at least in the early stages. When, however, failure occurred in both of a pair of cultures raised from the same parent culture of the previous generation, the lineage was allowed to lapse. Two lineages had lapsed by F_{10} in the eight descendants from each reciprocal cross in the first experiment, and one of the four from each reciprocal in the second experiment. More frequently, however, one culture of a pair failed, the two cultures for the next generation being then raised from the survivor. Twenty flies of each sex were counted from each culture and only cultures yielding this minimum were used. No weighting has been employed in the analysis to accommodate lost cultures or lineages. The numbers of cultures available in each generation are shown in table 3.

TABLE 3

Numbers of cultures in each generation of the two experiments. The descendants of the reciprocal crosses are shown separately

Generation	1	2
F_3	8+8	8+8
F_4	13+13	8+8
F_5	12+14	8+7
F_6	13+14	8+7
F_7	13+12	6+8
F_8	14+14	6+7
F_9	13+12	6+5
F_{10}	7+12	5+4

The various cultures of F_2 will contain comparable ranges of genotypes and so will not be expected to differ for genetical reasons in the estimates they give of V. This will no longer be true in F_3, since the genetical differences of the parents taken from F_2 will be reflected in the genotypes present in the F_3 progenies. We must

therefore expect the variation in V between cultures to be greater than random if bilateral stability is under genic control. The members of each twin pair of F_3 cultures will be genetically no more alike on the average than members of different pairs; but in F_4 and later generations this will no longer be true. Genetically produced differences in V should on the average be less between cultures of the same pair than between different pairs, and while under the breeding system used differences between pairs (*i.e.* between lineages) should increase with the generations, those within pairs should decrease. Finally, there should be a genetical correlation of offspring cultures with parent cultures from F_3/F_4 onwards if genic segregation is occurring. These experiments provide therefore a variety of tests of our conclusion that O and S differ in genes affecting developmental stability. The tests may not be intrinsically sensitive, as the genetical variation will be confounded with the high sampling variation of V, but the observations available should suffice to give some sign of genic segregation if it is taking place.

The values of V are given separately for the sexes in each generation in table 4. Data from the two experiments have been combined because their results proved to be homogeneous over the complete range of generations. It will be seen that V is lower in females than in males in every generation, and while the difference is significant in no single generation, it can hardly be doubted over the data as a whole. This difference is not an intrinsic property of the sexes as we can see from the parent stocks, but it is of course just as might be expected if sex-linked genes of the kind we have already come to suspect were segregating. It appeared that the sex-linked genes (assuming them to exist) favouring a lower variance were dominant, so that the males, which show the effect of sex-linked recessives more commonly than do females, should have a higher average V. The magnitude of the sex differences in F_3—F_{10} is compatible with the evidence from F_1 and F_2. The later generations thus add to the evidence which F_1 and F_2 provided for the existence of sex-linked differences between O and S. There is no evidence that the sex difference varies between cultures, experiments, crosses or generations ($\chi^2_{[269]} = 278{\cdot}000$, $P = 0{\cdot}35$), such differences as might be expected being presumably so small as to be swamped in the joint tests.

The sex-linked genes might also express themselves in another way. The cross O×S would bring in two dominants for low V from the mother to one recessive for high V from the father, while S×O would bring in two recessives to one dominant. There should thus be a higher proportion of recessive genotypes and hence a higher average V in the later generations of S×O than of O×S. This is indeed the case over all but two of the generations (table 4) and the results taken together strongly suggest that the offspring of S×O have somewhat higher V's than do those of O×S, so adding further

to the evidence for sex-linked effects. The difference in average V between the reciprocals is slightly smaller than that between the sexes, so that the two comparisons agree sufficiently well. An item for the difference between reciprocals has been taken out of all the analyses, so that the comparison between cultures should not be distorted by the reciprocal difference.

The pooled or average values of V are also shown for each generation in table 4. Since these involve the sexes and reciprocals equally (or very nearly so) in each generation, they may be fairly used for comparison. The values of $\overline{V}$ from table 4, together with

TABLE 4

Variances of the sexes and of the reciprocals

Generation	Females	Males	O×S	S×O	Pooled
F_3 . .	1·92	2·16	1·91	2·17	2·0389
F_4 . .	1·83	2·05	1·86	2·03	1·9436
F_5 . .	1·96	2·28	2·01	2·22	2·1212
F_6 . .	1·86	2·10	1·99	1·97	1·9789
F_7 . .	1·98	1·99	1·80	2·17	1·9852
F_8 . .	1·98	1·99	1·87	2·10	1·9852
F_9 . .	1·72	1·99	1·77	1·95	1·8561
F_{10} . .	1·84	2·01	1·95	1·91	1·9285
Average .	1·89	2·07	1·89	2·06	1·9797

those for O, S, F_1 and F_2 from table 2, are plotted in fig. 1. It will be seen that $\overline{V}$ rises under inbreeding until F_5, by which generation it has progressed a long way back towards the mid-parent, which it would be expected to approach asymptotically. After F_5, however, $\overline{V}$ falls and seems to become more or less stable a little above the F_1 value. This behaviour suggests the intervention of selective forces favouring a greater stability, and hence lower $\overline{V}$, than is shown even by the O parent. The failure of cultures and even lineages recorded in table 3 would appear to offer the opportunity for selective changes in $\overline{V}$ and one can hardly doubt that competition within culture bottles must also have its effects in maintaining heterozygosity at a higher rate than expected in the absence of selection. It is therefore not unreasonable to ascribe this unusual behaviour under inbreeding to the action of the " natural " selection which is to be expected in experiments such as the present ones. Whether the continuation of the inbreeding lines beyond F_{10} for a number of generations more comparable to the hundreds which O and S have undergone, would have resulted eventually in values of $\overline{V}$ more comparable to those of the parents must remain a matter for surmise.

The pooled values of $\overline{V}$ in table 4 supply us also with the estimates of sampling variation needed for the analysis of the differences in V

between cultures. This analysis has been undertaken by Steven's method. V is used as the variate and subjected to an analysis of variance, items being taken out for the sex difference and its interactions, and for the differences between experiments and reciprocals and their interaction, which comparisons have already been considered. The sum of squares remaining is for differences between cultures, and after F_3 this can be partitioned further into items for differences

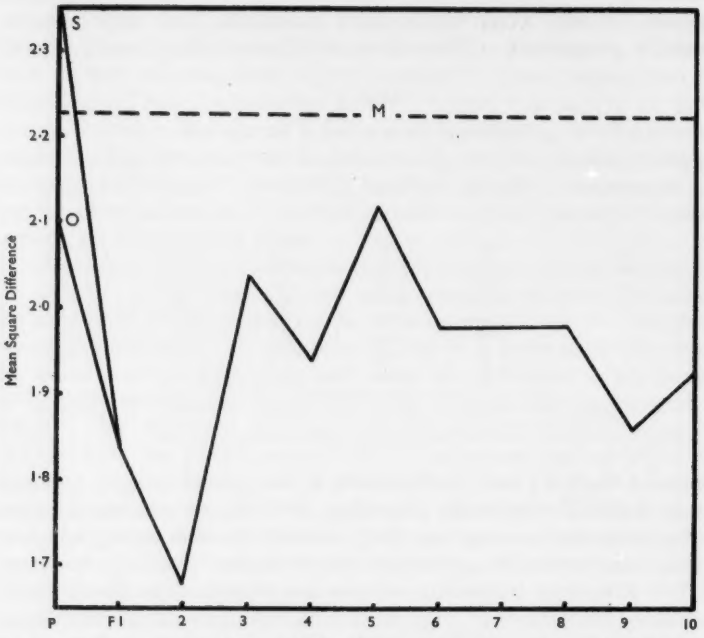

FIG. 1.—The mean square difference ($\bar{V}$) between the numbers of sternopleural chaetæ on the two sides of the flies in the parental lines, their crosses and the generations of the inbreeding experiments. The values shown are averaged over sexes, reciprocals and experiments. M is the mid-parent value, *i.e.* the average of the two parents, O and S.

between the twin pairs of cultures and for differences within these pairs. The sum of squares so obtained is divided by twice the square of $\bar{V}$ as estimated by pooling variation within cultures of the generation in question (*i.e.* $2\bar{V}^2$), and treated as χ^2s to give tests of significance of the culture differences. Since, however, the sum of squares rise with the numbers of degrees of freedom, the χ^2 values do not of themselves offer a simple means of assessing the size of the culture differences. Each χ^2 has therefore been divided by N, the number of degrees of freedom. When based on a reasonable number of degrees of freedom, the value so obtained will be close to 1 when the culture differences

are of the same size as the sampling variance. It will fall below 1 where culture differences are subnormal and rise above it where culture differences are greater than sampling variance would lead one to expect.

In the parents this measure of culture variation is about 1, and in F_1 and F_2 it falls below 1 (though not significantly) so that, as we have already observed, there is no evidence of culture differences exceeding the sampling variation expected on the basis of the variance within cultures, in the generations where comparison is between cultures of comparable genotypes. In F_3 and later generations on the other hand we should expect the measure of variation to exceed 1, as genic segregation should lead to the cultures being no longer comparable in genotype. Furthermore, the measure of variation for comparisons between twin pairs should tend to rise, though not very rapidly, as the genetic differences between pairs will tend to increase under the mating systems used, while variation within twin pairs should fall as inbreeding progresses.

The results of the analyses for all generations including parents, are given, in terms of our measure of variation, in table 5 and plotted in fig. 2.

TABLE 5

Variation between cultures, as measured when the sampling variation ($2\overline{V^2}$) relevant to each generation is taken as unity. The variation in the sex difference (i.e. *culture × sex interaction*) *is included for comparison. The number of degrees of freedom is shown in brackets*

Generation	Variation between cultures		Variation of sex difference
P	0·94 (48)		1·08 (48)
F_1	0·67 (48)		0·58 (48)
F_2	0·60 (38)		0·86 (38)
F_3	1·57 (28)		1·22 (28)
	Between pairs	Within pairs	
F_4	1·54 (20)	1·60 (18)	0·85 (38)
F_5	1·58 (18)	1·29 (19)	1·25 (37)
F_6	1·10 (18)	1·12 (20)	1·22 (38)
F_7	1·37 (18)	1·10 (17)	0·92 (35)
F_8	1·79 (17)	1·82 (20)	0·99 (37)
F_9	1·24 (16)	0·63 (16)	0·75 (32)
F_{10}	1·87 (13)	0·70 (11)	1·10 (24)

The variation in the sex difference of V between cultures is included in table 5 for comparison. This sex-culture interaction is not significant, so that its value oscillates round 1 in a random manner over the generations. It thus provides us with, so to speak, a random frame of reference with which to compare the values for variation between cultures. These do not exceed sampling variation until F_3, where the variation rises to 1·57, which is itself significant at the 5 per cent.

level. Thereafter, all the measures of variation between cultures are above unity, except for the item within twin pairs in F_9 and F_{10}. As we have seen, the variation within twin pairs should fall with the generations from a value in F_4 about equal to that between pairs (an expectation borne out by the data) to something approaching 1 in the late generations. Apart from a suprisingly, and no doubt

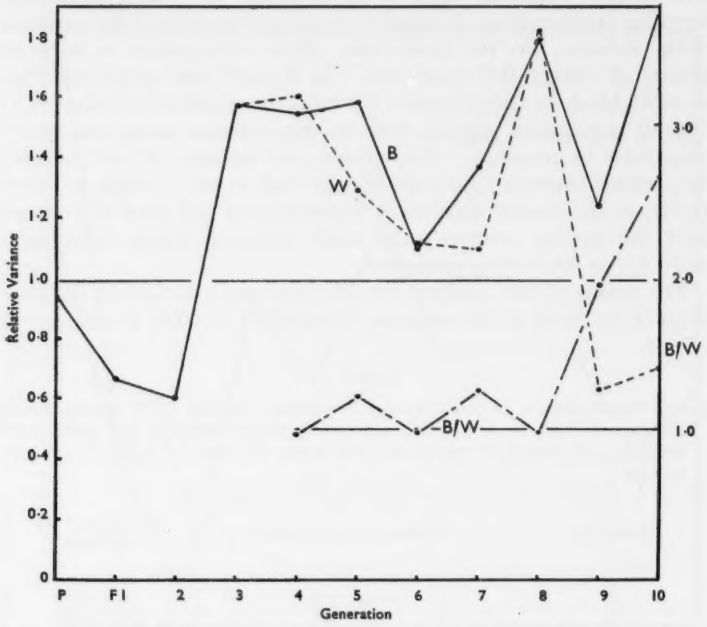

FIG. 2.—The relative variances between cultures ($2\bar{V}^2$ for the generation taken as unity) in the inbreeding experiments. After F_2 the relative variances are shown separately for comparisons between (B) and within (W) twin pairs of cultures. The ratio B/W is also plotted (scale on the right margin of the graph). Results are pooled over the two inbreeding experiments as these are homogeneous.

misleadingly, high value in F_8, the variation within pairs behaves as expected, the sub-normal values of F_9 and F_{10} being within the range of departure from 1 which the other data of the table, especially those from F_1 and F_2, show to be permissably ascribable to sampling variation.

The behaviour of the measure of variation between pairs is more erratic, but it certainly shows no steady fall and may indeed be regarded as compatible with the slow rise we should expect. Taken as a whole, the variation between cultures from F_3 on, is highly significant ($P<0{\cdot}0001$), so showing the expected contrast with the P, F_1 and F_2 generations. The mean value of the variation between

pairs in F_4-F_{10} (1·49) exceeds the mean within pairs (1·23), but the difference (though in the expected direction) is not fully significant. The ratio of the variation between, to that within pairs (B/W) should rise with the generations, as indeed it appears to do, even if a little erratically (fig. 2).

One further test of genic segregation remains to be applied, that of covariation between V in parent culture and offspring cultures. This can hardly be a sensitive test, for it must be obscured by the high sampling variation. Even so, however, taking the data of all generations together (*i.e.* pooling the covariances of F_4 on F_3, F_5 on F_4 and so on up to F_{10} on F_9), we find a positive regression of V in offspring culture on V in parent culture, the regression coefficient being 0·106. This is just significant at the 5 per cent. level when we take into account that it is positive as it must be if the correlation is genetic in origin. There is no evidence of any change in the value of the regression coefficient over the generations, but in view of the insensitivity of the test, we could not expect to detect such changes unless they were of a magnitude which could not readily be explained on genetical grounds.

Our many tests for the segregation of genes affecting the variance of the L−R difference have thus all been as successful as could be expected : our various expectations have been borne out reasonably well by the data. There can thus be little doubt of the existence of differences between the parent stocks, O and S, in respect of such genes, some of which furthermore appear to be sex-linked. The magnitude of the differences which segregation produces, seems however to be rather small. An inbreeding experiment, on the other hand, cannot be expected to tell us much about the magnitudes of differences which the extreme products of segregation can produce : response to selection should yield much more information. We shall therefore consider the effects of selection on V ; but before doing so we must examine a possible difficulty in the interpretation of the data presented so far, and one which is also relevant to the interpretation of results from selection experiments.

(d) The relation of asymmetry to chaeta number

So far we have assumed without discussion that V is a straightforward and adequate measure of stability. We know, however, that the number of sternopleural chaetæ (L+R) differs between the two lines O and S, and also varies among the families in the descendants of the cross. We must therefore ask whether change in L+R results in a direct developmental way in any corresponding change in V, and if so whether the relation so found would serve to explain the differences observed in V ; for if the changes in V merely reflected changes in the absolute number of chaetæ they could hardly be regarded at the same time as indicating genetic variation in the

control of stability as opposed to genetic variation in the magnitude of effect in development. We should expect that if such a relation exists, the higher the number of chaetæ, the more variable would be the manifestation, so that A = L+R and V would be positively correlated.

A number of considerations suggest that whatever the relation between A and V, it is not such as to make V interpretable wholly in terms of total chaeta number; that in fact the changes we see in V must be related at least in part to variation in the control of stability as opposed to magnitude of effect. In the first place, males

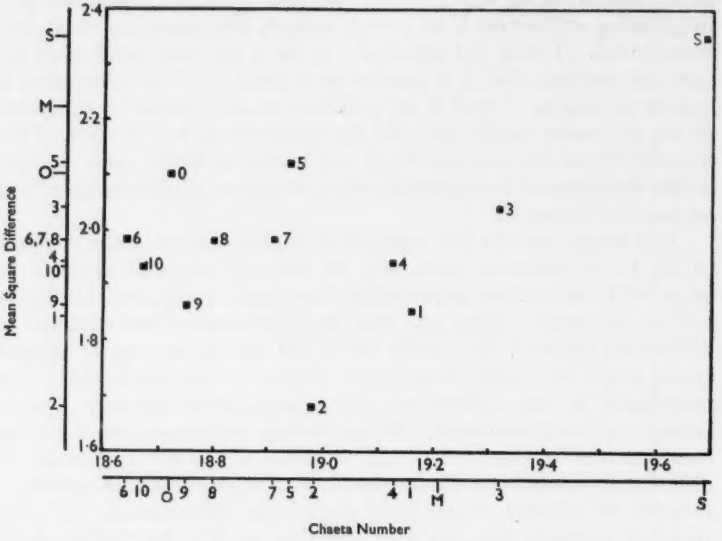

FIG. 3.—The relation of mean square difference (V) to gross chaeta number (A) for the different generations of the inbreeding experiments. The generation values of V are projected onto a single line on the left of the diagram and those of A onto a line at the bottom. M is the mid-parent value obtained by averaging the parents, O and S.

and females of O and S show similar values of V but differ in A; and at the same time A varies between cultures for environmental reasons, while V is homogeneous over cultures. The two inbreeding experiments were, as we have seen, homogeneous for the values they gave for V generation by generation, yet the second experiment regularly gave values of A about half a chaeta lower than did the first, possibly as a result of environmental differences. Furthermore, when we compare the mean values of A and V for the two stocks and for the various generations derived from their cross, we find little evidence of any simple relation between them (fig. 3).

The question has, however, been pursued further by finding the

regression of V on A within the stocks and their descendants and by making corresponding adjustments to the variation in V. All regressions were obtained by relating the value of V for a culture to the average value of A for that culture. If there is a correlation between A and V which is fixed by a developmental or scalar relation of the kind we are discussing, the regression so obtained should be equivalent to that resulting from comparisons within cultures. Characters under the control of at least partly separable genic (or environmental) systems could not, on the other hand, show such a fixed correlation. The regression found from comparisons within and between cultures would differ, and a genic correlation, even where linkage was involved, must vary as we move from the parents through the various generations of their crosses.

In estimating the regression of V on A, V has been calculated round a mean of zero for L—R. This departs from our procedure of earlier sections and, as we have already seen, is not completely justifiable, because the mean of L—R sometimes does depart a little from O. The departures of the means from O are so small, however, that the inaccuracy introduced thereby cannot be such as to invalidate our interpretation of the regression analyses.

TABLE 6

Regression of V on A

Parents	O	S	
	−0·04	0·75	
	O×S	S×O	Pooled
F_1	−0·02	0·07	0·02
F_2	0·42	0·00	0·19
	Expt. 1	Expt. 2	Pooled
F_3	0·41	0·32	0·35
F_4	0·22	0·34	0·27
F_5	0·28	0·28	0·28
F_6	0·32	−0·03	0·12
F_7	0·26	0·18	0·22
F_8	0·26	0·10	0·19
F_9	0·18	0·15	0·16
F_{10}	−0·11	0·21	0·06

Overall regression F_2-F_{10} = 0·20

The regressions of V on A are given in table 6, the values being shown separately for the two reciprocals in F_1 and F_2, and for the two experiments in F_3-F_{10}.

The most striking feature of the table is the variation in the regression of V on A. One parent, O, shows no evidence at all of

a relation, while the other, S, has a regression both highly significant and positive as would be expected. The difference between these two parental regressions is itself fully significant ($P = 0{\cdot}01$-$0{\cdot}001$). The regression in the case of S must be developmental, so that we are immediately faced with the situation that there are genetic differences in the developmental correlations themselves. Thus stability as measured by V can be related to absolute chaeta number, but it can also be divorced from it by genetic adjustment.

The reciprocal F_1s agree in showing little if any relation between V and A, being like the O parent in this respect. The reciprocal F_2s differ somewhat from one another, though not quite at a significant level ($P = 0{\cdot}07$-$0{\cdot}06$), one showing a relation of V and A, the other giving no evidence of any such relation. The later generations in both experiments show regressions intermediate between those of the parents. Taken by themselves, the regression in F_6 differs significantly at the 1 per cent. level between the two experiments, but this is the only such case and indeed if all the generations are considered together, the experiments agree perfectly well. No weight can therefore be given to the discrepancy in F_6, or, perhaps, to the difference between the reciprocal F_2s either. One trend is however clearer. There is a fall in the regression from F_3 to F_{10} which is significant at the 5 per cent. level at least. Such a fall in the relation between V and A would suggest that the correlation they show in these generations is at least in part due to linkages which become progressively resolved with the generations, and the absence of correlation in F_1 accords with this view. Certainly the relation between V and A revealed by these regressions is not the simple one that would be expected to invalidate the use of V as a measure of stability in development.

The effects of the regressions have, however, been investigated still further by adjusting the variation in V from culture to culture to allow for the relations observed. Using the culture means as the variants and taking all deviations from the generation mean, the sum of squares of V (*i.e.* $S(V^2)$) is corrected for the linear regression on A by subtracting $\frac{S^2(AV)}{S(A^2)}$ from it. One degree of freedom is used up in making the correction, which leaves a sum of squares measuring the variation in V unrelatable to the variation of A in that generation. The residual $S(V^2)$ can be divided by twice the square of the mean value of V in the generation ($2\overline{V}^2$) to give a χ^2 testing the heterogeneity of V over the cultures as described earlier. The value of $\overline{V}$ used in finding the divisor should itself be adjusted for relation to A, but this would involve calculating regressions inside the cultures and has not been done. The divisor may therefore be somewhat too high, so that the χ^2s may be too low, and the evidence for heterogeneity of V somewhat underestimated. The χ^2s have also been divided by

the corresponding numbers of degrees of freedom to give a measure of variation between cultures relative to $2\bar{V}^2$ taken as unity. The results of these tests are given in table 7. The two experiments have been pooled in the tests of F_3-F_{10}, and the two reciprocals in those of F_1-F_2, it having been shown by analyses of covariance that only F_4 and F_7 give differences between experiments verging on significance when the generations are taken individually, and that taking all generations together there is no indication of such differences.

TABLE 7

Differences in V between cultures after adjustment for regression on A

	χ^2	N	P	Relative variation between cultures
O	21·79	23	0·55	0·95
S	13·10	23	0·95	0·57
F_1	32·63	48	0·95	0·68
F_2	25·90	38	0·93	0·68
F_3	42·74	30	0·06	1·43
F_4	56·54	40	0·04	1·41
F_5	51·29	39	0·09	1·32
F_6	40·21	40	0·47	1·01
F_7	49·29	37	0·08	1·33
F_8	65·99	39	0·04	1·69
F_9	38·51	34	0·28	1·13
F_{10}	37·82	26	0·06	1·46
F_3-F_{10} pooled	382·39	285	0·001-0·0001	1·34

The picture given by this table is essentially the same as that obtained earlier when no allowance was made for any relation of V to A. There is no evidence of heterogeneity of the cultures in O, S, F_1 and F_2, where it is not expected, but good evidence in the generations from F_3 to F_{10}. Perhaps when taken individually, no one of these generations can be regarded as unambiguously revealing culture differences; but all tend in the same direction, all but two have probabilities of less than 10 per cent., and when the χ^2s are pooled the significance is beyond dispute. The values shown in the last column for the relative variation between cultures are a little lower than those of table 5, in which relations between A and V are neglected. This is to be expected for, as we have seen, the divisor $2\bar{V}^2$ has not been adjusted for the regression. Even so, all generations from F_3 onwards show values greater than 1, while all earlier generations give values less than 1. Despite their underestimation, the differences between cultures show clearly in the generations where segregation would lead us to expect them, and are absent where segregation would not be effective.

Thus in large measure the variation in V cannot be referred to a

direct developmental relation with A : it can be altered independently of A, so that, in this sense at least, we have no reason to question the use of V as a measure of stability. We shall have further evidence of the separability of V and A from the results of selection, to which we must now turn.

3. SELECTION EXPERIMENTS

(*a*) *The first experiment—Main lines*

Both selection experiments commenced from crosses between the O and S stocks. The crosses were made reciprocally using single pairs of males and females. F_2s were raised from these F_1s, again using single pair matings. Selection began in F_2 (generation S-0), the two highest females being chosen out of twenty counted as mothers of the first high selected generation (S-1) and the two lowest females as mothers of the first low selected generation, and similarly with the males. The method of selecting parents was the same in later generations except of course that only high flies were taken in the high line and only low ones in the low line. Six F_2 cultures were used, four from S×O and two from O×S.

The experiment was divided into two parts. In one, originating from one S×O and the O×S crosses, the high males and females from one F_2 culture were mated to give one high culture in S1, and similarly for the low line. This closed mating system was practised throughout, so that each line, high and low, comprised three separate sub-lines, each consisting of one culture per generation, with four parents, two of each sex. In the other part of the experiment, there were again three cultures in each line, high and low, in each generation with four parents per culture ; but the females taken as parents from culture A were mated to the males from B, the females from B to the males from C, and the females from C to the males from A. This system was varied after S-19 by reducing the number of cultures per line per generation reduced from three to two, but mating was still continued between cultures. This cyclical mating system will maintain a higher outbreeding and heterogeneity than the closed sub-line system, but the inbreeding of the inbred line will not of course itself be as great as if single pairs of full sibs had been used as parents.

After mating for a day or two in tubes, the parents were moved into the customary half-pint culture bottle and allowed to lay there for two days before removal. All counts were made on offspring from these bottles, except in a few cases where the first bottle having failed, flies were taken from a second culture in which the parents were placed after removal from the first bottle and in which they stayed for six or seven days.

A word is necessary about the rigour of selection. This was based

on the difference in sternopleural chaeta number between the left and right sides irrespective of sign. Sometimes, especially in the high lines, there was no doubt which flies to take as parents, two having higher differences than all others. Sometimes, however, one fly would have a greater difference than any other, to be followed by a group of several with the same next highest difference. One of this group would then be taken at random for use with the outstanding individual as joint parents. Where the highest difference was shown by a group of several flies, two were taken at random as parents. No cognisance was taken of the sign of the difference.

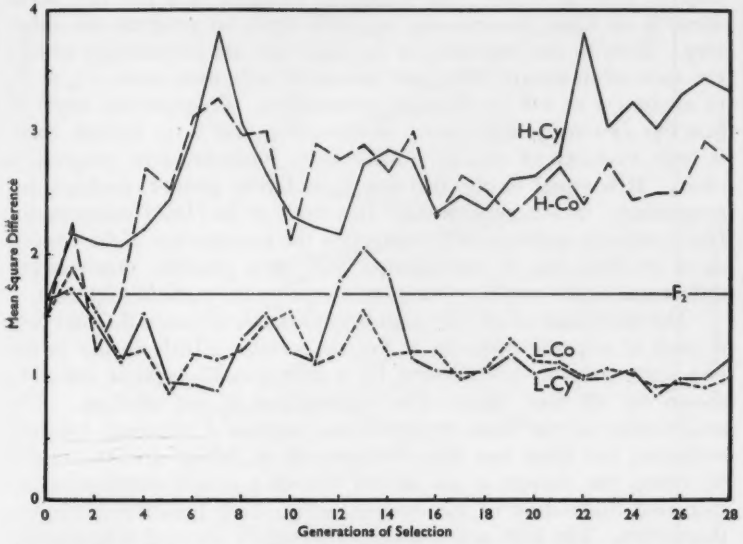

FIG. 4.—The changes in mean square difference between the sides (V) in the first selection experiment. F_2 is taken as generation S-0. H indicates lines selected for high difference and L those selected for low difference. Cy indicates the cyclical and Co the closed mating systems, as described in the text.

In the low line it was almost invariably the case that more than two flies showed the lowest possible difference, viz. equality on the two sides. Two were then taken at random for use as parents. As selection became effective in the low line, a rising proportion of flies showed no difference between the sides, so that the rigour of selection necessarily fell. No such consideration applies in the high line, of course.

The results of selection in both closed and cyclical lines are shown in fig. 4. The difference between the sides is measured by V taken about zero : no correction was made for the departure of the mean from O. Each point is the average of observations from three cultures,

except in the relatively few cases where one or, even more rarely, two of the three failed, and, of course, after S-19 in the cyclical lines, where only two cultures were raised.

Several features of the figure are obvious. There is clear evidence of response to both high and low selection in both closed and cyclical lines, so that there can be no doubt of the existence of heritable variation. The general behaviour of the lines also suggests polygenic variation, as will be discussed in more detail later. The response is less in the low than in the high lines, partly, doubtless, because of the falling rigour of selection in the low line, and partly also, one assumes, because there is in any case a limit to progress in a low line while there is, at least theoretically, no such limit on progress the other way. Even so the responses in the high line are surprisingly small; the root mean square difference advanced only from some 1·3 in F_2 to about 1·7 or 1·8 in the later generations. Progress was rapid at first but extremely slow later. Between S-9 and S-19, indeed, there is little evidence of change. After S-19, however, slow progress is clear. It is equal in the two low lines but is greater, perhaps not surprisingly, in the cyclical high line than in its closed counterpart. Circumstances unfortunately compelled the termination of the experiment at S-28, but if continuation had been possible, much larger differences might well have been produced in the cyclical high line.

The behaviour of all four lines is remarkable between S-6 and S-9. A peak of response is shown at S-7, or perhaps a little earlier in the low lines, only to be followed by a retrogression against selection shown by all four lines. The explanation is not obvious. The simultaneity of the four retrogressions suggests a common external influence, but since two lines retrogressed by falling and the others by rising, the change is not merely one of a simple stabilisation or increased fluctuation of the environment. Two possibilities suggest themselves. The high and low genotypes might respond differentially to a given environmental change. Though possible, this hardly seems likely, since the difference postulated would be one not merely of magnitude but of direction of response.

The other possibility depends on the correlated response that is often shown by fertility to selection for other characters. Fertility, as measured by the average number of flies hatching from the three culture bottles, fell sharply with the early responses to selection in all four lines (fig. 5). Now if, as was evidently the case in the selection lines of Mather and Harrison (1949), the chromosomes carrying the combination of genes of more extreme effect on the L—R difference also carried the combinations of genes causing greater reduction in fertility, any relaxation of selection would lead to retrogression of the chaeta character as the fertility character took charge (see Mather and Harrison). Should, therefore, the environment have become less stable in its effects on the chaeta difference between S-6 and S-9,

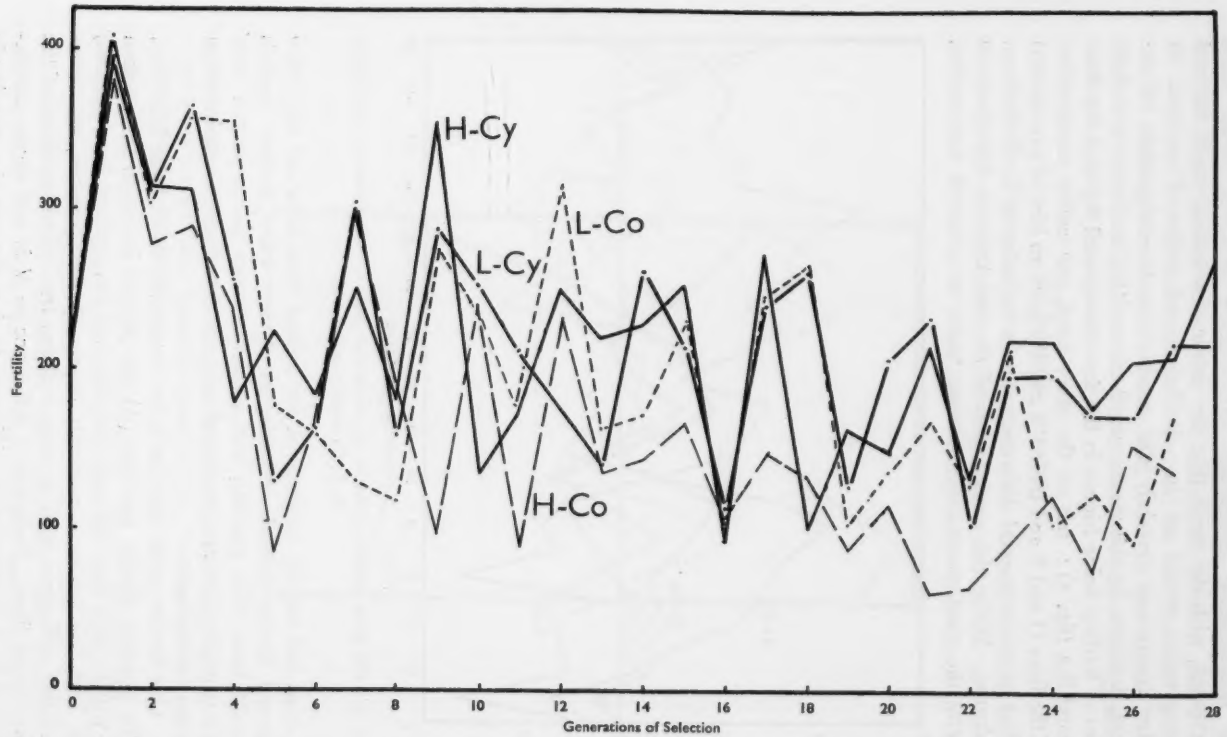

FIG. 5.—Fertility, as measured by average output per culture of all flies, in the four lines of the first selection experiment. H, L, Cy and Co as in fig. 4.

the non-heritable component of variation in L—R would have increased, with the result that the rigour of selection would fall and retrogression would set in. Deliberate relaxation of selection in later generations certainly did result in such retrogression of the lines, doubtless by this means (see Section 3 (*b*)), and there is a slight hint of fertility being higher in the four lines round S-8 and S-9 than round S-5 (fig. 5) ; but on the other hand, our earlier examination of the lines O and S and their F_1s and F_2s gave no hint of any marked effect of environmental differences on the variation of L—R between cultures. While this explanation of the simultaneous retrogressions seems the most reasonable, as being based on a known mechanism,

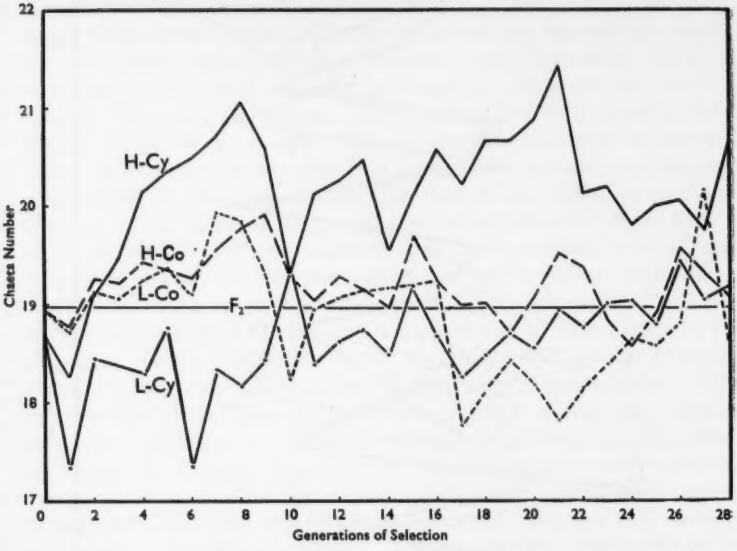

FIG. 6.—The gross number of chaetæ (A) in the four lines of the first selection experiment. H, L, Cy and Co as in fig. 4.

it must still remain conjectural, for neither these lines nor any other showed similar behaviour again, at a time when further analysis would have been possible, and no opportunity has presented itself of attempting to induce retrogression by the experimental manipulation of the environment.

One further point remains to be mentioned about these four lines. The absolute chaeta numbers (A) are plotted in fig. 6, from which it will be seen that although the high and low cyclical lines differed, the high line having a markedly higher absolute number, no corresponding difference is to be seen between the chaeta numbers of the two closed lines. Evidently a difference in V is not always accompanied by a corresponding difference in A : the one is not a simple

secondary effect of the other. Indeed even in the cyclical series an analysis of covariance shows that the difference between the V in the high and low lines cannot be accounted for by reference to the relation shown between this variance and A within the lines. Thus these four selection lines bear out the conclusions from the regression analyses of the previous section.

(*b*) *Relaxation of selection*

At the S-22 generation two new lines were started, one from each of closed high and low lines respectively. No selection was practised in these new lines, but in other respects they were maintained in the

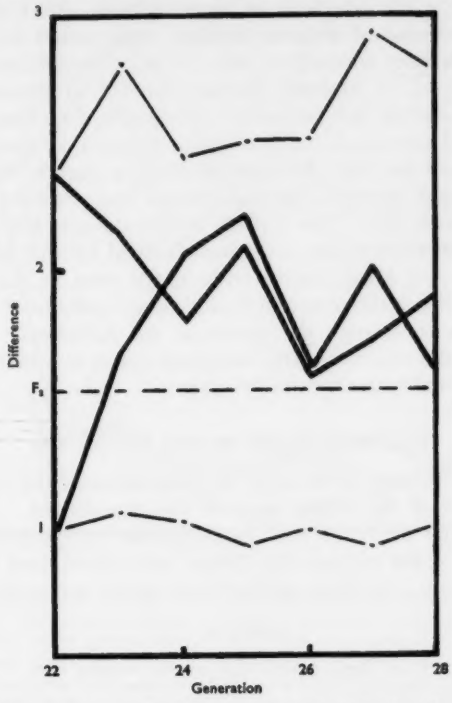

FIG. 7.—The effect on mean square difference of relaxing selection (heavy lines) in the first selection experiment. Relaxation began at S-22. The selection lines (light) are also shown for comparison.

same way as those from which they sprang. In two generations the two new lines had come together in respect of their mean square chaeta differences (fig. 7), albeit at a level slightly above the average value shown by F_2s. Thereafter the unselected lines continued together, with that from the low line slightly, and probably insignificantly, above its fellow.

The small excess of the level at which they stabilised over that of F_2 should probably not be emphasised; the significant feature is that they came together, and did so in only two generations, despite the twenty-two generations of selection in opposing directions to which their ancestors had been subjected. Clearly the selection had not succeeded in rendering the high and low lines homogenic for the combinations of genes producing the more extreme, and therefore favoured, phenotypes. In fact, genic combinations similar in effect to those of the F_2 from which the selection lines arose must have persisted in these lines, or at least combinations from which recombination could readily build up others similar in effect to those of F_2. Given that the combinations of more extreme effect also gave the correlated response of reduced fertility, they would be held in the high and low lines themselves only by the selection, and would be eliminated from, or at least sharply reduced in frequency in, the new lines where, by the relaxation of selection for chaeta difference, fertility would become the capital character and take charge of events.

The relaxed low line did seem to show a slightly higher fertility than its selected ancestor, but no obvious improvement occurred in the relaxed high line. The fertility results do not, therefore, supply quite the confirmation one could have wished for this interpretation, but they are not incompatible with it, for even in the case of the relaxed high line a difference in fertility may easily have been present large enough to change the genotype yet sufficiently small to be obscured by the relatively large sampling errors to which the present estimates of fertility are inevitably subject.

(c) *Crosses between the high and low lines*

Reciprocal crosses were made at S-18 between the high and low selection lines of the closed part of the experiment. F_1s and F_2s were raised and new high and low selections were started separately from the F_2s of the reciprocals. These were maintained by the same methods as the parent high and low lines except that only two cultures

TABLE 8

V in Crosses between high and low selection lines

		F_1		F_2	
		Females	Males	Females	Males
Crosses	H×L	2·18	2·03	2·83	1·45
	L×H	1·43	0·98	1·95	1·80
Parents	H	2·15	2·92	2·65	2·00
	L	1·12	1·02	1·40	1·25

were used in each generation of each line instead of the three of the parent lines.

The most striking feature of these crosses was the difference between the reciprocal F_1s and F_2s. The values of V in F_1 and F_2 are set out for males and females separately in table 8, together with corresponding figures for flies of the parent lines raised at the same time.

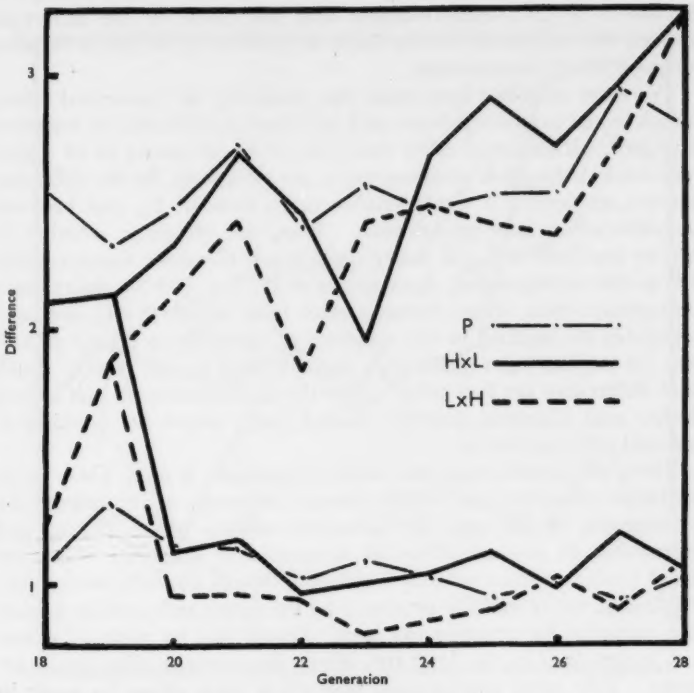

FIG. 8.—Mean square difference in the crosses between the high (H) and low (L) selection lines in the first selection experiment and its behaviour under selection from these crosses. The parent selected lines (P) are shown for comparison. The crosses were made between flies from S-17, so that F_1s appear at S-18, F_2s at S-19 and the derived selection lines from S-20 onwards. H×L and L×H each give rise to an F_1, an F_2 and two reciprocal lines, one selected for high and the other for low V.

The difference between the reciprocal crosses is clearly shown in this table, as it is also in fig. 8, where, however, the mean of the sex values is plotted. The crosses fall nearer to the parent lines from which the mothers were taken, so that two possible explanations offer themselves: that the reciprocal differences may be due to effects of sex-linked genes or that they may be due to an effect of the mother in partially determining the phenotype of her offspring by extra-

nuclear means. If sex linkage is the cause, the difference in F_1 should be between the males from the reciprocals, and in F_2 between the females. Such differences do exist (table 8) but there are similar, though admittedly smaller, differences between the females of F_1 and the males of F_2 where, on the basis of simple sex linkage, we should not expect them. Thus, while sex linkage may play a part, and indeed we have already had reason to postulate sex-linked genes (Section 2 (*b*)), it seems unlikely that the whole of the difference between the reciprocals can be fairly attributed to the effects of genes borne on the X chromosome.

We must therefore keep open the possibility of a maternal effect, though on the present evidence such an effect could hardly be regarded as proved. If maternal effect there should be, it cannot be of a kind traceable to permanent plasmagenes in the cytoplasm, for the difference between reciprocals is much smaller in F_2 than in F_1, and becomes even smaller in later generations. True, the offspring, whether in high or low line, of $L \times H$ never quite reach the same average value of V as the corresponding descendants of $H \times L$; but the discrepancy is no greater than occurs between sister lines within $H \times L$, and may reasonably be ascribed to the accidents of sampling in taking parents from the original heterogenic high and low lines for the crosses. Such small differences are inevitable under these circumstances and indeed Mather and Harrison describe similar cases where no question of maternal effect enters in.

Thus, the present maternal effect, if genuine, is more likely to be ascribable either to some direct somatic influence of mother on the development of the egg, an influence exerted before laying and comparable to uterine effects in mammals or maternal effects on eggs in birds ; or to a relatively long lived, though not fully permanent, cytoplasmic entity initially produced by the genes and possibly having some capacity for reproducing itself, though not to such an extent as to maintain it in the long run where the corresponding genes are absent. This latter explanation, which has been shown to apply in other cases in snails, *Drosophila* and elsewhere (Mather, 1948*a*), would obviously account not only for the reciprocal difference, but also for its gradual diminution and eventual disappearance.

The difference between the reciprocals is not, however, the most important feature of these crosses. The two reciprocals agree in responding to selection so quickly that the levels of the parental lines both high and low are reattained by only two generations selection, though it took at least five generations of selection to achieve those levels in the original building up of the parent lines. This is the same phenomenon as was observed by Mather and Harrison (1949) in selecting for abdominal chaeta, and the explanation is presumably the same. The first selection is effective by building up, through recombination, new linked polygenic combinations of more extreme

effect, which once built up, tend to behave as units or "effective factors" in inheritance. Following crossing of the H×L selection lines, these combinations segregate out as effective units, with the result that the genotypes of the parental lines reappear almost at once, so permitting selection to restore the parental levels much more quickly than they were reached when first built up. It would thus appear likely that the genetic system controlling stability in develop-

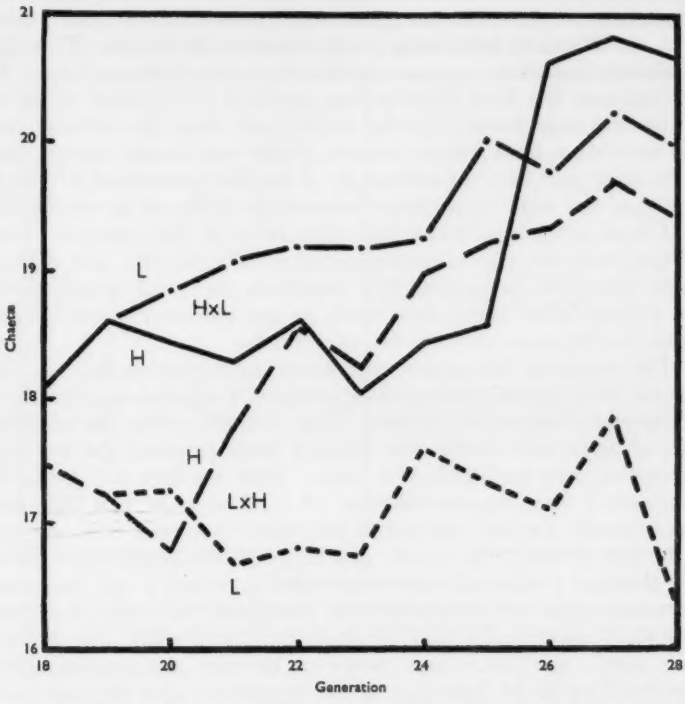

FIG. 9.—Chaeta number in the crosses H×L and L×H and their derived lines selected for high (H) and low (L) mean squares differences. Compare with fig. 8.

ment is similar in properties to those which Mather and Harrison discussed.

One further point needs mention before leaving these crosses. The absolute numbers of chaetæ in them and the selection lines to which they gave rise are shown in fig. 9. Though the high and low lines from H×L and L×H clearly resemble both one another, and the parent H and L lines, in V, they depart widely in A, thus emphasising once again the lack of dependence of difference on absolute number.

(*d*) *The second selection experiment*

A second selection experiment was started, again from the F_2 of the cross O × S, and proceeded to S-13 before it had to be terminated. This experiment was divided into two parts, in one of which each culture was raised from a single pair of parents and in the other from four parents, two of each sex on the pattern of the first experiment. Three lines were maintained in each part, one by high selection (H), one by low selection (L) and the third by the use of unselected parents (U). For this third line the parents were taken at random : they were not chosen as being near to the mean of the family. Thus the unselected line offers a picture of the effect that drift can have. In the high and low lines selection was practised at the same rigour as in the first experiment : in the double pair lines the extreme two flies were taken from twenty counted of each sex in each culture, and in the single pair lines the extreme fly of the first ten counted, although a further ten were then always counted to bring up to twenty the total from which was found the value of V in that culture. Two cultures were set up in each generation of each line, the mating system being of the cyclical type described, except of course where one culture failed. The time spent by the parents in each culture bottle was the same as in the first experiment.

The results of this second experiment are set out in figs. 10. In general, these results confirm those of the first experiment, and show no especially remarkable feature. After hesitant starts, the selection lines of single and double pair matings made progress, the low line responding more rapidly in both cases. Both low lines stabilise fairly soon near a mean square difference of 1. The single pair high line rose to nearly 3 at S-7 and stayed just below that value until the end of the experiment. The double pair high line rose until S-10 when it had obtained a value of rather more than 3, though it fell somewhat thereafter. The two unselected lines fluctuated, the double pair line somewhat more sharply than its single pair counterpart, but in both cases they came to occupy positions between the corresponding selection lines in the later part of the experiment after the high lines had showed their responses. Evidently, as indeed could hardly have been doubted, it is not reasonable to ascribe the behaviour of the selection lines to drift.

The fertility of the single pair lines fell a very little during the course of the experiment, perhaps due to the slight inbreeding which would go on even under the mating system used. The fall could in any case hardly be correlated response to selection for it occurred as much in the unselected line as in the selected. The double pair lines showed no clear evidence of a fall in fertility. It is perhaps surprising that fertility should hardly be affected in this second selection experiment while falling so strikingly in the selection lines of the first. The difference may be due to the absence from the

second experiment of the very sharp responses seen in the early generations of the first, responses which were not equalled even in the latter generations of the second. Albeit that these gains in the first experiment were substantially lost after a few generations, as already discussed, the effect on fertility seems already to have been established (fig. 5). In any case, it should be a characteristic of correlated response due to linkage that it will appear or not appear according to the nature of the linkages and the recombination which breaks them. Such a difference would, however, be troublesome to explain by a correlated response due to pleiotropic action.

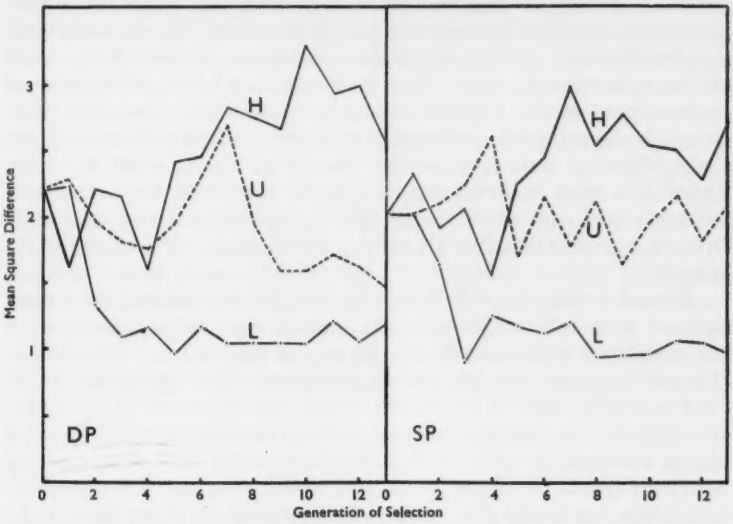

FIG. 10.—The effect of selecting for mean square difference in the second selection experiment. H indicates selection for high V, L selection for low V, and U the unselected lines. The double pair mating (DP) and single pair mating (SP) parts of the experiment are shown separately.

One last point remains to be made about this second experiment. In both single and double pair selections the value of A in the unselected line departed more from the high and low lines than did these two selection lines from one another. Thus, once again, we can see how the bilateral difference can be separated from gross chaeta number, so that change in the one cannot be a mere secondary expression of difference in the other.

4. THE GENETICAL STRUCTURE OF CONTROL

These results, from both inbreeding and selection experiments, leave no doubt that there is a heritable component in the control of the difference between the two sides of *Drosophila melanogaster* in this

cross between the O and S lines. The genic structure of this component is, however, less obvious. The very complexities of the character, stability in the development of an individual, virtually precludes the observation of simple segregations except such as would have the grossest effects. There is an indication that the X chromosome carries at least one gene affecting the character, in that the males of reciprocal F_1s and the females of their F_2s show some difference (table 2). At the same time, there seems also to be an autosomal element, in that all the classes of F_1 and F_2 offspring have lower bilateral variances than do their two parent lines. Again, selection produces lines with higher values of V than the parents, so that genotypes capable of transcending in their effects that of the parents can be obtained, presumably by recombination. Furthermore, when selection is relaxed, these lines revert to the levels of expressions characteristic of the unselected crosses, so that these selection lines cannot be homogenic : they must each carry a number of homozygous types, differing from one another and from the parents, or, more likely, they must be heterozygous. And if the latter, they cannot be heterozygous in the same way as the F_1, for they differ in expression from it, one selection line showing a much higher V and the other a lower.

Beyond seeing that inheritance is most likely complex, we cannot proceed with full confidence. We cannot even set a minimum to the number of genes, or effective factors, in the system. Nonetheless, it is worth noting that the general behaviour of the character in this cross resembles that of characters which can be shown to be under the control of a polygenic system, such as the number of abdominal chaetæ analysed by Mather and Harrison (1949) and Harrison and Mather (1950). Both are characters which vary in wild type flies, and which can confer fine shades of adjustment on those flies. Both are characters in which selection can easily bring about expressions transcending those of the parental lines, or, in other words, both are characters for which the genotype carries potential variability. Selection for the bilateral difference is perhaps less easily, or at least less strikingly, effective than selection for abdominal chaetæ, even in the upward direction where there is no technical limit set to its rigour ; but even in this connection, it should be noted that in the cyclical high line of the first experiment, selective advances were still in progress at S-28. The difference between the two characters may thus lie more in the difficulty of releasing the potential variability than in its quantity.

It is worth noting too that following the H×L and L×H crosses, selection restored the parental levels of the bilateral difference in only two generations, whereas these levels themselves took at least five generations of selection to establish from the original crosses of O×S and S×O. Though less marked, this is as we have already

observed, reminiscent of the difference in speed of response to selection between the offspring of the original crosses and the offspring of crosses between selected lines in Mather and Harrison's experiments; a difference which they showed to be most reasonably attributed to the effects of linkage. The slow advance under selection in the last few generations of the high cyclical line adds its weight to the evidence for linkage effects. We also have a similar relation of the selected character to fertility, in that when selection for the bilateral difference was relaxed, the character reverted to the unselected level in both high and low lines.

Control by a polygenic system of the kind which Mather and Harrison discussed will thus account for the properties of the bilateral difference in the crosses and the selection lines. It will also account for the difference in the expression of this character between the O and S lines and their immediate crosses. Where polygenic variability expresses itself in wild individuals, the balance of the genic combination comes to be adjusted by natural selection, so that in outbreeding species, such as *Drosophila melanogaster*, inbred lines have a different and usually less advantageous expression of the character than do crossbreds. In the present case, a higher bilateral difference, or lower stability, would presumably be less advantageous, so that the higher variances of the inbred lines, O and S, could be regarded as manifestations of inbreeding depression, an explanation which Mather (1946 and 1950) has already suggested for the higher variability of floral characters within individuals of inbred lines of *Primula sinensis*.

A remarkable feature of the present results is that the variance is lower in F_2 than in the F_1 of the cross. Two explanations might be advanced. As Dr J. M. Thoday has pointed out to me, the genic balance of the F_2 individuals from a wide cross might resemble those of flies from wild populations more closely than would the balance of F_1. Wild individuals will necessarily be homozygous for a proportion of the genes varying in the population, since the maximum proportion of heterozygotes which outbreeding can give will, in general, exceed by little, if any, the proportion resulting from random mating. Thus the F_1 of a wide outcross might be too heterozygous to give the best genic balance, so that its F_2 would have a more advantageous expression of the character.

The second possible explanation is that the bilateral difference may be partly a maternal character and hence influenced by the balance of the mother's genotype as well as by that of the individual itself. The lesser variance of F_2 would on this view reflect the better genic balance of F_1. The difference between the F_1s of the $H \times L$ and $L \times H$ crosses also suggests such a maternal effect, as we have already observed. Our present results, however, offer no way of deciding finally between these explanations.

5. STABILITY IN DEVELOPMENT

We have discussed stability as it is manifested by the differences shown by a bilateral structure within individuals, because such a character is technically convenient for the investigation. Properties in respect of stability will, however, affect the variation between individuals in the expression of single as well as bilateral structures. Properties of stability are less easy to investigate by comparisons between individuals, however, because such comparisons must reflect both segregation for genes and the action of external agencies affecting the average expression of the character, as well as the effects of genes controlling stability round that average.

A comparison of F_1s with their parent lines, where these latter show but little genetic variation within themselves as compared with the genic differences between them, and where all are grown under a comparable range of conditions, can nevertheless provide relevant evidence. The variation in flower morphology between the plants of an F_1 from the cross *Petunia axillaris* × *violacea* is less than the variation between the individuals of each parental stock, even on a scale where the average expression in F_1 is almost at the mid-parent value (Mather, 1949). This finding has been extended by Robertson and Reeve (1952) to certain bodily measurements of *Drosophila*, where F_1s are less variable between individuals than are the parent inbred lines, when a " coefficient of variation " is used to allow for differences in mean expression. In both cases, the F_1s, though heterozygous, will be as homogenic as their parents, and since the individuals of both parental and F_1 families will be showing the effects of environmental agencies, we must conclude that the individuals of F_1 are less responsive—less buffeted about—by these agencies. The genotypes of the F_1s give a more stable development better buffered against upset by outside factors.

The bilateral differences we have observed in the present experiments may reflect the effects of local differences in external conditions affecting the sides of the fly. No evidence was, however, obtained of the differences between cultures that one might perhaps expect to find, at least within the parent lines, if the bilateral difference in the character were in large measure dependent on, and an expression of, differences in external conditions to which the two sides of the fly have been exposed. It would seem likely, therefore, that within the fly the bilateral differences reflects the effects of internal upsets in development rather than external inequalities ; and that the greater stability of some of the generations and lines displayed a greater genotypic capacity of these flies to correct their own internal accidents.

All these species, *Petunia*, *Primula* and *Drosophila*, which we have observed to display greater stability in the more crossbred individuals,

are outbreeding forms. As we have already pointed out, this is understandable if greater stability is selectively advantageous, so that it would characterise those cross-bred genotypes which had been exposed to natural selection and whose balance would therefore have been adjusted to produce just this effect, rather than the more inbred genotypes which must be rare in the wild. Two consequences would follow on this view. In the first place, we should expect smaller differences in stability between inbred and cross-bred families of naturally inbreeding species, though some differences may remain as relics of a past ancestry of outbreeding. Observations on naturally inbreeding plants, at present not available, will be needed to take this point further.

The second consequence is that we should expect the levels of stability achieved in respect of different characters to vary with the selective properties of the character. Thus, while gross differences in the manifestation within an individual of almost any bilateral character could hardly fail to be deleterious, some instability leading to small bilateral differences in a character such as number of sternopleural chaetæ, would be unlikely to bring a serious disadvantage to the fly; and indeed we have seen that some bilateral variation remained even in lines selected for its reduction. This is most probably true of a great many characters of the individual. Wing size and shape are, however, likely to be more critical, for even quite small differences between the wings on the two sides would be expected to have a marked effect on the properties and power of flight. The variation in wing length shown by an inbred line of *Drosophila melanogaster* has been analysed by Reeve and Robertson (1953) who find that the coefficient of variation (*i.e.* ratio of standard deviation to mean length) ascribable to asymmetry is 0·48 per cent. In the F_2 of the cross between Oregon and Samarkand the $\overline{V}$ when averaged over reciprocals and sexes was 1·6843, or on the basis of single sides 0·8422. The standard deviation due to asymmetry is thus 0·918, which when compared with the single side average of 18·98 chaetæ (table 1) gives a coefficient of variation of 4·84 per cent. Although the F_2 shows the lowest asymmetry for sternopleural chaeta number, the coefficient of variation is 10 times as high as that found for wings.

In discussing stability from the point of view of the effect of rectification of upset (whether arising from external or internal cause), we must not lose sight of possible causes of increase of the bilateral difference other than the mere reduction of ability to cope with such upsets. Where difference reflects only the ability or lack of it to adjust upsets, an increase in the average difference between the sides merely marks an increase in the general variation of their behaviour. But if the two sides interact with each other, by physiological means which at present we need not discuss, so that when on the average the character was high in expression on one side it was correspondingly

low on the other, a large average difference between them could be associated with smaller chance upsets on the individual sides than would be the case if interaction was absent. Such an interaction is of course most obvious when one side is regularly the larger, so that a consistent bias exists between them ; but it might also exist without leading to a bias of this kind, where either side could take the lead, the other then following in its expression, by virtue of the interaction.

An interaction of this kind, unfixed in respect of side, would be impossible to detect by bias, for insofar as each side had an equal chance of taking the lead, it could not lead to an average excess of one side over the other, nor could it be detected by a consideration of variation in the expression on a given side, for this would reflect not merely the variation intrinsic in development once the course of the side in question had been laid down, but would also contain a component reflecting the uncertainty that the side would follow the one course or the other. It should, however, be detectable from the properties of the bilateral difference when measured without taking sign into account. When sign is neglected, the average difference no longer measures bias, and should rise *pari passu* with the mean square or root mean square difference, if both merely reflect the variation due to upset ; but if an interaction comes into play between the sides, the mean should be increased disproportionately.

Such an interaction would give rise to what Timoféeff-Ressovsky (1943) has called *antisymmetrical manifestation*, for which he was unable to select effectively in the case of his *vti* gene. There is, however, some evidence that this relation has been favoured in our lines selected for high difference between the sides. In fig. 11 the standard deviation of the difference is plotted against the mean difference, both taken neglecting sign, for the parents O and S, the F_1-F_{10} generations of the inbreeding experiment, and the H, L and U lines of the selection experiments. The reciprocal F_1 and F_2 crosses are plotted separately as are the F_3-F_{10} generations from the two inbreeding experiments, in order to bring out the error variation to which the points are subject. Four H and four L points are shown, as found from the last ten cultures raised in each of the four H and L lines, two from each selection experiment. The two unselected (U) points are also shown from the second selection experiment.

The points fall into three groups, the parental, inbred and unselected, the low selection and the high selection. The parental, inbred and unselected group of points and the group of four L points fall very nearly on a common straight line passing through the origin, as would be expected if the standard deviation changed proportionately with the mean. The standard deviation, and with it the variance, is lower than the mean, but there seems to be no reason to anticipate it to be otherwise, as there is no ground for expecting these differences to follow a Poisson distribution.

When, however, we turn to the four H points, while they too agree sufficiently well with a linear relation among themselves, they clearly fall on a line different from that describing the parent, inbred, unselected and low points. The mean is higher for a given standard deviation or variance, just as would be the case if selection had favoured the rise of an interaction between the sides. Either the existing genetic system gives a new developmental relation beyond a certain point past which selection has carried it, or perhaps more likely a new genetic

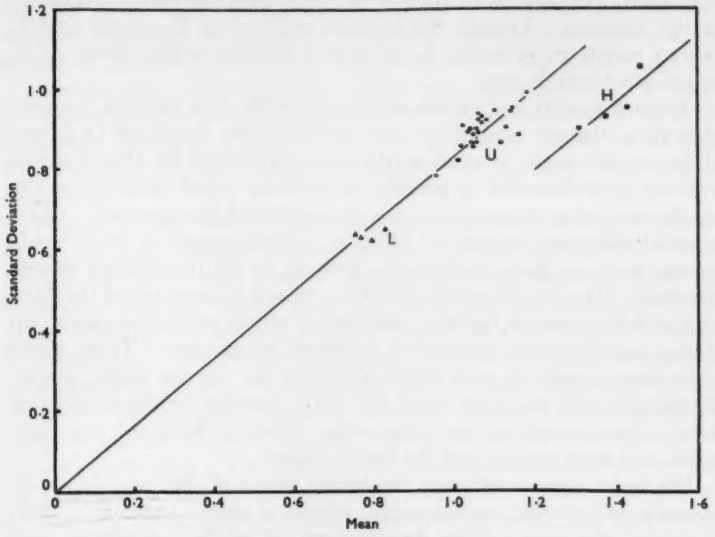

FIG. 11.—The standard deviation of the difference between the sides plotted against the mean difference where both are found neglecting the sign of the difference (*i.e.* neglecting whether the difference was in favour of left or right). The four low selection lines, two from each experiment, are represented by the four points in group L, and the four corresponding high selection lines in group H. The U, or unselected group, includes points for the parent lines, O and S (1 point per parent), for their reciprocal F_1s and F_2s (1 point per reciprocal per generation), for the generations F_3-F_{10} in the two inbreeding experiments considered separately (1 point per generation per experiment) and for the two unselected lines of the second selection experiment (1 point per line). The lines are suggested regression lines, that for the U and L groups being drawn to pass through the origin and that for the H group being drawn parallel to its fellow.

system has been established which gives this new relation. However this may be, the relation in the H group appears to differ from that in the L and U groups in that the two sides follow different paths in development, which side following which path seeming to be a matter of chance, but each side varying no more by upset from its path, once this is laid down, than did the sides round their common path in the ancestral flies.

The difference which appears to have become thus fixed in these experiments is small, but an extension of the process would presumably give a situation such as is observed, for example, in male fiddler crabs. In these animals, I am kindly informed by Dr I. Gordon of the Natural History Museum, the claw is always much larger on one side than on the other, but the large one is equally often on right as on the left. In *Uca mordax* Dr Gordon has counted a ratio of 41 : 34 for the large limb on the right and left respectively, in *U. tangeri* a ratio of 10 : 13, and in *U. inversus* 14 : 16 among the animals available at the museum. Though the animals ascribed to *U. mordax* and *U. inversus* might conceivably be of mixed specific origin, those of *U. tangeri* are certainly not.

Once a system had developed which leads to a marked bilateral difference, though one which was equally often displayed in favour of the one side as the other, it would not seem difficult for a mechanism, whether environmental or genetic, to grow up which determined the regular favouring of one side in the asymmetrical development. Quite a trivial difference should be able, at an early stage of what would appear to be so finely balanced a process, to tip it regularly in one direction. The result would then be a biased asymmetry of the kind with which we are so familiar, and which might well have sprung up in this way from an unfixed or unbiased asymmetry. There seems to be an example of such fixation among the hermit crabs, where, Dr Gordon tells me, it is usual for whole families or sub-families to show a bias towards a particular side, which is, however, the right in some of these groups and the left in others.

We have been considering the laying down of the two (or more) channels of adjusted development within a single individual, but, of course, the cases where development of whole individuals may follow one or other of the possible adjusted paths implies the same capacity of the basic genotype to offer these paths as alternatives. In these cases, however, the result will be, not an asymmetry within the individual, but a polymorphy between individuals, of the kind we commonly see in relation to crossbreeding devices like dicœcy or heterostyly, or in other characters such as the " phase " difference in locusts. The switching system may depart in these cases from that which operates in asymmetry, and will in general be expected to come to depend on a genetic segregation, or a distinct environmental factor. But even so the successful operation of the system must require the selective adjustment of the common genotype to offer the alternative channels of adequate development, an adjustment which we have seen broken down by overwide crossing or intense inbreeding in various plants and animals (Mather, 1948*a*), and whose building up under selection we may now have observed in its very early stages in *Drosophila*.

6. SUMMARY

The canalisation of development leading to characteristic differences between individuals and between parts of the same soma requires not only that initially small differences between cells and parts of cells can be built up to give large effects, but also that similar differences appearing in other cells or at other times can be reduced to relative ineffectiveness. The part played by the genotype in securing this stability in development can be investigated by the study of asymmetry and its variation in bilateral structures which show little or no difference between the average expressions of the two sides.

The number of sternopleural chaetæ may differ markedly between the two sides of an individual *Drosophila*, but there is only a very small difference between the sides when averaged over a number of flies. Furthermore, this small bias varies with sex, genotype and culture conditions.

The difference between the sides is more variable (and development hence less stable) in the Oregon and Samarkand inbred lines than in the F_1s and F_2s of their crosses. Two inbreeding experiments, started from these F_2s and carried on to F_{10}, gave evidence of segregation of genes affecting the stability of development as revealed by variation in asymmetry. Some evidence appeared of a sex-linked component in this genetic control. No evidence was obtained of environmental differences in stability between cultures. Natural selection within cultures appears to favour a higher degree of stability than that shown by the parent lines. The differences in stability could not be regarded as springing solely from differences in overall number of chaetæ. In fact, the relation of variation in asymmetry to variation in overall chaetæ number itself appears to change with genotype.

Two selection experiments, started from the same cross, confirmed the genetic control of stability by establishing differences between lines selected for high and low expressions of asymmetry. The differences between the high and low selection lines were themselves not large, but selection appeared still to be effective when the longer experiment had to be terminated after 28 generations. Again the change in asymmetry could not be referred to change in overall number of chaetæ. One of the experiments gave evidence of a correlated response in fertility and of the building up of linked polygenic combinations by the selection practised.

Though it was impossible to establish complete proof, the genetical system mediating stability appears to be polygenic in nature. There may also be a maternal effect. The level of stability appears to depend on a genic balance, itself the product of natural selection, in the species of *Drosophila*, *Primula and Petunia* in which it has been investigated. The properties of asymmetry should thus vary between inbreeding and crossbreeding species (a comparison on which we have

no information at present) and between characters of the same species, as indeed appears to be the case.

In the lines selected for increased asymmetry there is evidence of an interaction having arisen between the two sides of the fly in development, such as could produce the type of extreme, but directionally unbiased, asymmetry of the male fiddler crabs ; and which, by the likely next step of directional fixation, could give rise to the directionally biased asymmetry of the hermit crabs.

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THE ROGUE TOMATO : A PROBLEM IN NUCLEAR, CYTOPLASMIC AND ENVIRONMENTAL CONTROL

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I. INTRODUCTION

THE tomato, *Lycopersicon esculentum*, in its native region, Peru, is cross-pollinated to the extent of 50 per cent. (Rick, 1950). In England the cultivated tomato is a greenhouse plant which has to rely entirely on self-pollination. The English varieties, therefore, are the result of selection for total self-fertilisation, and in consequence have been inbred for varying numbers of generations according to the age of the variety. When grown in the open ground English varieties do undergo some cross-pollination ; the occasional practice of raising seed out-of-doors would mean that a small amount of crossing would have occurred at some time in the life of a variety. But in general the varieties are highly inbred and true-breeding.

There is, however, an off-type or rogue which has segregated for many generations in several varieties. This off-type has been given many names such as " Jack ", " Feather Leg " and " Rogue ". The latter term has come into general use and is adopted here. I shall use the term in a specific sense for the one type of plant to be described and not in the general sense of any segregating off-type which occurs in a cultivated crop. The rogue plant has occurred sometimes as a rare mutant but also in 15 per cent. of seedlings in several varieties, and a frequency of 50 per cent. has been recorded.

In the U.S.A. tomatoes have been selected for cultivation in the open ground and have not attained the same degree of self-fertilisation as English varieties. It is remarkable that rogues have not been seen in the U.S.A. varieties.

Crane (1939) bred from normal and rogue plants of the variety Balch's Fillbasket, but he could find no consistent difference between the two in the proportion of rogues produced in the progeny. He also showed that the rogue condition was not transmitted by grafting. Darlington (1944) on the grounds of irregular segregation and similarity with the rogue pea (Bateson and Pellew, 1920) put forward a theory of cytoplasmic control.

One other feature of the rogue tomato was the great difference in numbers of rogues segregating from samples of the same seed sown at different times. This strong environmental effect supported Darlington's hypothesis. With this background and a stimulus from the findings of Sonneborn in *Paramecium* that the reproductive rate

of kappa particles in the cytoplasm is dependent upon temperature, I took up the problem with a view to testing the effect of environment with particular emphasis on temperature. If the environmental factors could be found and controlled then the problem would be resolved into elucidating the genetic control of a frequent segregant in an inbred line.

2. MATERIAL

Ailsa Craig.—This is an old commercial variety which has regular bilocular fruits. The original seed was obtained from Sutton & Sons in 1940 and has been maintained from greenhouse-grown plants. Before the present experiments started single plants had been self-pollinated for 2 generations and during the experiment for a further 6 generations.

Leafmould Resister.—This variety has been derived from crosses involving at least three varieties. Seed was obtained in 1940 and has been maintained from greenhouse-grown plants.

Recessive stock, d p s o r y. This stock was obtained in 1930 and has been rigorously inbred for at least 20 generations.

Lycopericon pimpinellifolium (yellow fruited). The origin of this stock is obscure but it is known to have been inbred for at least 4 generations before its use in the present work.

I am indebted to Mr Crane for the original seed of Ailsa Craig, Leafmould Resister and the recessive stock *d p s o r y*.

3. DESCRIPTION OF ROGUE PLANTS

The earliest difference between the Rogue and a Normal plant can be seen from 16-22 days after sowing, according to the temperature. At this stage the cotyledons are fully expanded and the first true leaves can just be seen. The normal seedling has the first leaf much larger than the second leaf, the rogue has the two leaves of about equal size. The terminal segment of the first leaf of the normal is much larger than the other segments of the leaf; while in the rogue the segments of the first leaf are about equal in size. The leaves at this stage also have a different poise: those of the rogue appear flatter than those of the normal plant. Some of these differences can be seen in the photographs, plate I.

As the plants develop, other differences become manifest. The internodes of the rogue are much shorter, the leaves are smaller and with fewer segments. The first inflorescence frequently aborts, and side shoots develop much more freely in the rogue, *cf.* photographs, plate I.

The general effect, the short internodes, the smaller leaves and the development of side shoots, is characteristic of an unbalanced auxin system resulting in loss of apical dominance.

The rogue characters persist throughout the life of a plant. But Crane (1939) has reported a rogue plant of the variety, Best of All, which produced more normal growth during development. In the present experiments rogue plants have not been seen to change to

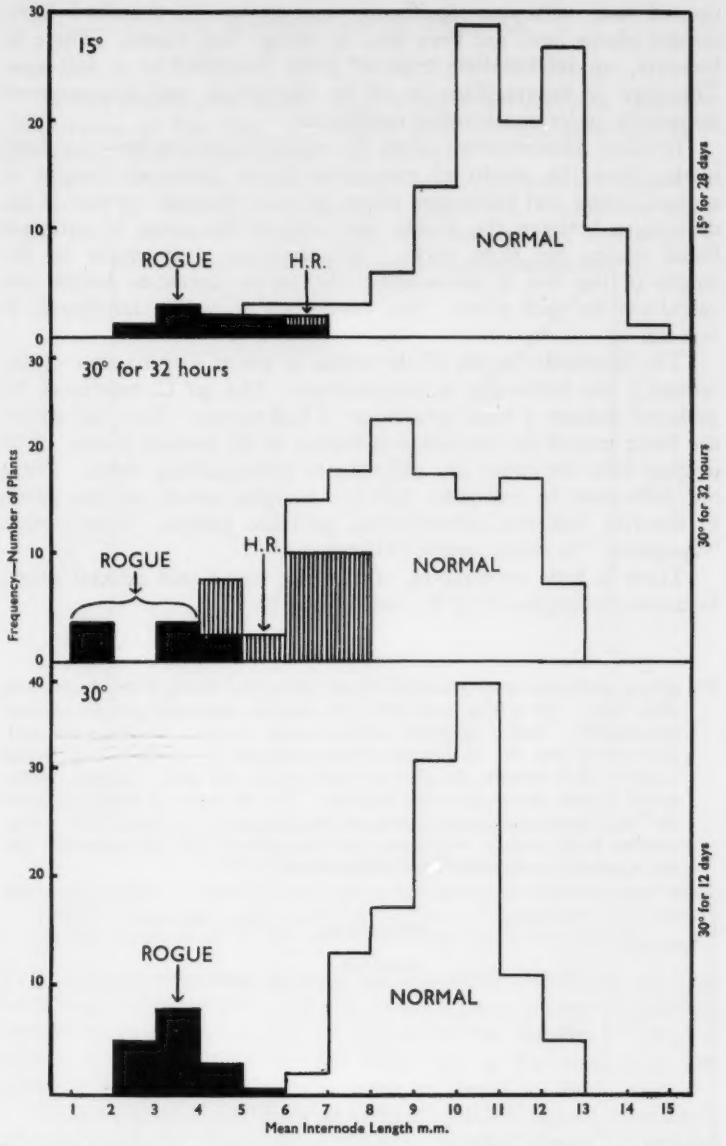


FIG. 1.—Mean internode lengths plotted against number of plants to show differences between the rogue, half-rogue and normal under three different germination treatments. The first 8 internodes were measured 45 days after cotyledon expansion: variety, Leafmould Resister. Note the high proportion of half-rogues at 30° for 12 days.

normal and, with one significant exception to be described later, normal plants have not been seen to change into rogues. There is, however, an intermediate type of plant described as a *half-rogue*. This type is intermediate in all its characters, and has occurred frequently under uncontrolled conditions.

Detailed measurements of all the rogue characters have not been made, since the results of measurements on internode lengths of normal, rogue and half-rogue plants give an adequate picture of the differences between the plants, and also of the range of variation found within the three types. Measurements were made on the length of the first 8 internodes; the mean internode length was calculated for each plant. The results are shown as histograms in text fig. 1.

The internode length of the rogue is about a third that of the normal; the half-rogue is intermediate. The 30° C. treatment for 32 hours induces a high percentage of half-rogues. They fall within the lower end of the normal distribution of the normal plants. This overlap does not cause any difficulty in distinguishing them. There are differences in leaf poise and leaf margins which are too subtle to describe but are, nevertheless, infallible guides. Their whole "syndrome" is characteristic of the rogue.

There is little overlapping of the true rogue and normal plants in internode lengths at 15° C. and none at 30° C.

TABLE 1

The effect of temperature on the production of rogue plants from normal parents in the variety Ailsa Craig. The seedlings were kept at the controlled temperatures until the cotyledons had expanded. Tests of significance on the percentages of rogues transformed into angles (last column) show that all differences between treatments are significant except between 2 and 3. Each treatment was given to 2 boxes of 100 seeds each. The plants received natural daylight through glass-sided incubators. For the purpose of temperature control the "day" period was fixed at 14 hours and the night period at 10 hours. The seedlings remained in the controlled temperatures until the cotyledons had fully expanded. This was a period of 14 days at 26°, and 28 days at 12·5°.

Treatment	Temp.		Germination	Rogues (per cent.)		ϕ
	Day	Night	actual nos.	on no. sown	on no. germ.	
1	26°	26°	96	11·0	11·4	19·8
			97	14·0	14·4	22·3
2	26°	12·5°	89	6·0	6·7	14·9
			94	8·0	8·5	16·9
3	12·5°	26°	87	7·0	8·0	16·4
			86	7·0	8·0	16·4
4	12·5°	12·5°	91	0·0	0·0	0·0
			82	3·0	3·5	10·7

4. ENVIRONMENTAL CONTROL

(I) *Means of transformation or re-orientation*

(a) *On progeny from normal plants of high-rogue producing varieties.*—The results of four heat treatments given during germination to the variety Ailsa Craig are given in table I.

Tests of significance show that all the comparisons are significant except 2 : 3. The high temperature treatment, 1 produces about eight times as many rogues as the low temperature treatment, 4.

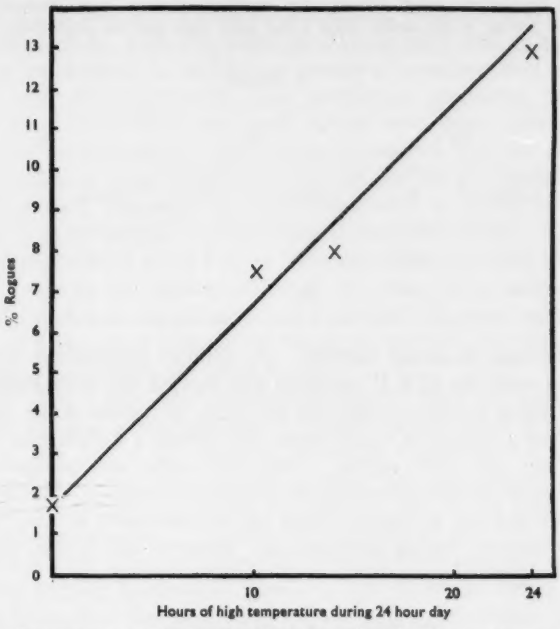


FIG. 2.—Percentage of rogues in Ailsa Craig plotted against the number of hours of heat treatment in each day : data extracted from table 1. Note the linear proportionality between the percentage of rogues and the number of hours of heat treatment.

It is immaterial whether the heat treatment is given during the dark or light period. For the results show that there is a linear proportionality between the percentage of rogues and the number of hours of heat treatment, despite the fact that one of the treatments was given in the dark and another in the light, see fig. 2.

Heat treatment was given to another *H.R.P.** variety, Leafmould Resister, and the results were 6·6 per cent. of rogues at 14° and 14·1 per cent. at 26°. The effect is similar to that with Ailsa Craig but not so marked, and for this reason the later work was concentrated on Ailsa Craig.

* *H.R.P.* High-rogue-producing.

(b) *On progeny of rogue plants.* Seeds from 3 rogue plants obtained from Ailsa Craig were germinated at 30° and 14°. The results are given in table 2. The effect of high temperature on rogue production is similar to that obtained with seed from normal plants (table 1). The difference between the 3 rogue parents in their rogue production, and the fact that a rogue parent can give fewer rogues than a normal parent will be discussed after the genetic control has been described.

TABLE 2

Percentage germination (actual numbers) and percentage of rogue plants produced from rogue parents of the variety Ailsa Craig under high and low temperature

Temp.	Parents					
	R_1		R_2		R_3	
	Germ.	R.	Germ.	R.	Germ.	R.
30°	91	4·4	80	6·3	75	14·6
14°	84	0·0	85	0·0	74	1·3

(c) *Low-rogue-producing varieties.* A similar experiment to that carried out with the H.R.P. varieties was carried out with Potentate, a variety which has given only 0·5 per cent. of rogues in the routine sowings over a period of many years and Stonor's Exhibition, which has given 0·1 per cent. rogues. Only one rogue was produced out of the 400 seeds of Potentate sown for the four treatments, thus showing that the proportion of rogues could not be increased in this variety by heat-treatment during germination. Stonor's Exhibition, another L.R.P., also yielded a negative result with heat treatment.

(ii) *Process of transformation*

(a) *Germination and rogue production.*—It is important to determine whether the higher production of rogues by the heat-treatment is due (i) to the transformation of a potentially normal seed into a rogue seed or (ii) to the "rogue seeds" needing a higher temperature for germination than the "normal seeds." The increased number of rogues at a high temperature would then be simply due to increased germination.

The results in table 1 do show a tendency for higher germination at higher temperatures but a regression coefficient calculated on the percentage of germination and the percentage of rogues was not found to be significant.

Table 2 shows quite inconsistent relationships between germination and rogue production. For example with Rogue No. 3 although the

germination was 75 and 74 per cent. in high and low temperatures respectively the corresponding percentages of rogues was 14·6 and 1·3. Furthermore with Rogue No. 2 a higher percentage of rogues was found with a lower germination, while with Rogue No. 1 the opposite relationship was found.

The simplest explanation is that the rogue seedlings produced by heat treatment are actually induced by the temperature, a conclusion which is emphasised by the next experiment.

(b) *Temperature-sensitive period.*—Temperature treatments in the first series of tests were extended to the time when the cotyledons were fully expanded. This was about 28 days at 12·5° and 14 days at 26°. In one experiment in which two groups of seedlings had been given 12·5° and 26° respectively until cotyledon expansion, the groups were then sub-divided and each batch was again treated at two different temperatures. The second treatment had no effect: the first treatment alone determined the proportion of rogues produced, showing that, although the rogue condition is labile in the early stages of germination, it soon becomes absolutely stable.

An experiment was set-up to determine more precisely the sensitive period within the first labile stage of germination. Seeds of two H.R.P. varieties, Ailsa Craig and Leafmould Resister were sown at 14° and given short periods (32 hours) at 30° at 6-day intervals from sowing. The results are given in fig. 3.

In Ailsa Craig, the experiments reveal that the most sensitive period for true rogue induction is about 18 days after sowing at 14°. For at this period the 32-hour treatment transformed 6 per cent. of the seedlings into rogues; this is about half the transforming effect of high temperature given for the whole of the period from sowing to cotyledon expansion. The first heat treatment given at sowing time and the last treatment at 24 days after had no effect on rogue production.

The variety Leafmould Resister shows a similar trend to that of Ailsa Craig but the most sensitive period for true rogue production is 12 days after cotyledon expansion. The period also in Leafmould Resister seems to be less sharply differentiated because all the heat treatments raise the frequency of rogues.

But the most interesting and unexpected effect of the short treatments in both varieties is the induction of the intermediate plants—the *half-rogues*, which have been described earlier. In the respect of half-rogue induction the two varieties show a similar pattern of response: half-rogues are produced by all short heat treatments and a maximum at the 18-day treatment.

Half-rogues have appeared from time to time in normal routine sowings (Crane, 1939). They are as stable as normal and rogue plants and do not revert as they get older to normals or rogues. They are a distinct class. Under normal greenhouse conditions of germination short periods of high temperature would be fairly common, and

these would account for the occurrence of half-rogues. It is significant in this respect that no half-rogues are produced when the temperature

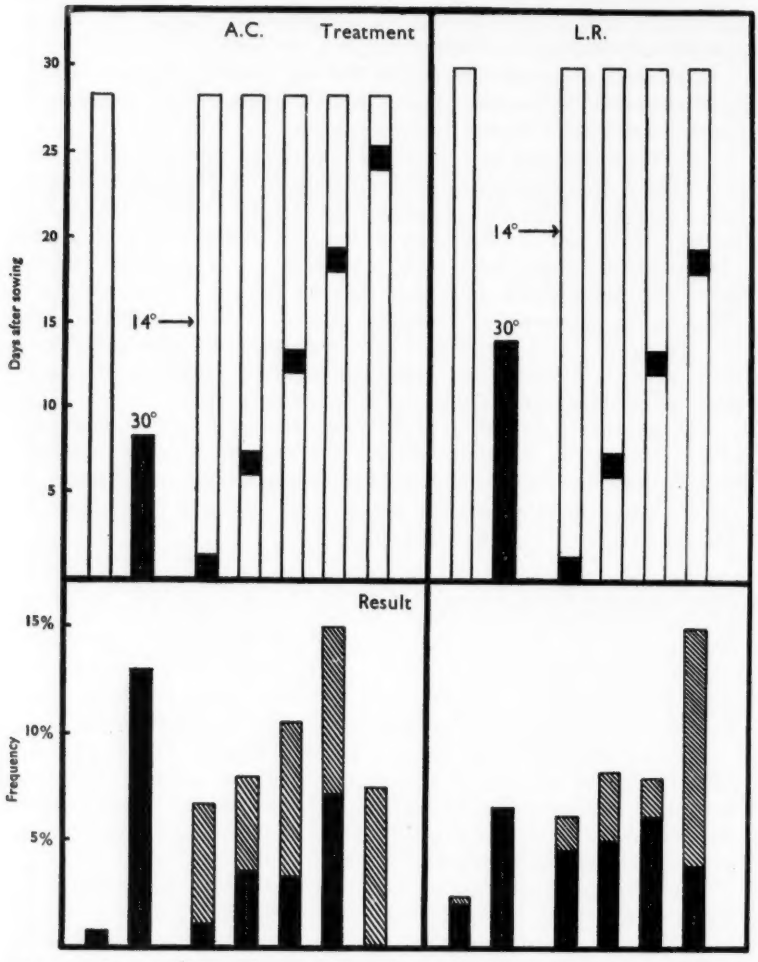


FIG. 3.—The results of experiments to determine the sensitive period during germination for the heat treatment effect. Above : heat treatments for 32 hours given at six-day intervals (indicated ■) are compared with heat treatments given for the whole of the germination period. Below : the frequency of rogues ■ and half-rogues ▨. Note the high proportion of half-rogues produced by the short heat treatments, and the most sensitive period at 20 days. A.C., Ailsa Craig : L.R., Leafmould Resister.

treatment, whether high or low, is given for the whole of the germination period.

The fact that some of the short heat treatments, which increased the proportion of rogues, were given after germination, excludes the explanation of the temperature effect on a basis of differential germination of the rogue and normal. Furthermore the half-rogues produced only by the short heat treatments are desicive evidence for an actual transformation or re-orientation from normal to rogue.

(c) *Day-length and light intensity at germination.*—In the course of several years it had been noticed that fewer rogues were produced in summer than in winter sowings. As the temperature of sowing in a warm greenhouse would be of the same order in winter and in summer, other environmental differences had to be sought. Light intensity and duration, showing as they do a wide variation throughout the year, were obvious factors to investigate.

An experiment using the variety Ailsa Craig and a constant temperature of 30° was designed to show the effect of light. The light was natural daylight in June, the seeds were sown on 11th June and the different treatments were continued until 19th June. Thus the day-length would be almost at its maximum and the light intensity was high during this period. There were four treatments (table 3).

TABLE 3

Shows the effect of reducing the day-length and light intensity on rogue production. Seed of Ailsa Craig at 30° C.

Day-length	Light intensity	Number of plants	Rogues
1. 16 hours . .	Normal	91	6·6 per cent.
2. 7 ,, . .	,,	185	15·6 ,,
3. 16 ,, . .	Half Normal	93	12·9 ,,
4. 7 ,, . .	,, ,,	170	11·7 ,,

1. *Control.* Natural day-length (approx. 16 hours) and natural intensity.
2. *Reduced day-length.* A 7-hour day obtained by covering the seedlings.
3. *Reduced intensity.* Intensity reduced to half by covering with muslin, natural day-length.
4. *Reduced intensity and reduced day-length.* Combination of treatments 2 and 3.

It will be seen that the control gives about half the percentage given by the same variety grown at the same temperature in the short days and low light intensity of February. Reducing the day-length and intensity separately have doubled the frequency of rogues to 12 or 15 per cent., which is comparable with February results. The combination of reduced day-length and light intensity do no more.

(iii) *The developmental effect*

Selfed seeds collected from different inflorescences of a normal plant of Ailsa Craig were sown separately in two lots ; one lot of each

at 30° C. and the other at 14°. The number of rogues produced from the first four inflorescences are given in table 4.

TABLE 4

Shows the effect of inflorescence on the production of rogues by normal plant of Ailsa Craig. Sown February 1948

Inflorescence	30° C.		14° C.	
	Germ.*	Rogues	Germ.*	Rogues
1	94	15·9 per cent.	90	5·5 per cent.
2	85	14·1 ,,	92	3·6 ,,
3	95	20·0 ,,	87	11·4 ,,
4	88	25·0 ,,	91	9·0 ,,

Analysis of variance on angles.

	$S(x-\bar{x})^2$	D.F.	M.S.	t	p
Between temps. . . .	203·011	1	203·01	8·499	<0·01
Between inflorescences . .	74·124	3	27·06	3·102	0·02-0·01
Error	8·434	3	2·81		

* Actual numbers out of 100 seeds sown.

There is a trend from low-rogue-production by the 1st and 2nd inflorescence to a higher production by the 3rd and 4th. An analysis of variance on the percentages transformed into angles shows that the differences between the inflorescences are on the verge of significance, $p = 0{\cdot}02\text{-}0{\cdot}01$.

A further test was made using the seed from three different normal plants, and recording the rogues produced from the first five inflorescences. The seed was sown at 14° in randomised positions in a glass incubator. The results of this test showed the same trend from low in the 1st and 2nd to high in the 3rd and 4th with a drop in the 5th.

The analysis of variance for this test showed that the effect of inflorescence was not statistically significant and the error, which is composed mainly of inflorescence-plant interaction, is very high.

The high inflorescence-plant interaction was due to the position of the maximum which in some plants was at the 3rd inflorescence and in others at the 4th.

Still a third test was made on seed from two other normal plants, and again the same trend was found.

Although none of the tests taken separately gave a significant effect for the inflorescences, taken together there can be little doubt of the genuine reality of the increase from 1st and 2nd inflorescence to the 3rd or 4th according to the plant.

Fig. 4 shows the combined results of the tests on plants which were scored for five inflorescences. If all the data from all tests are combined the mean figure for the 1st and 2nd inflorescence is 8·05 and for the 3rd and 4th, 13·42.

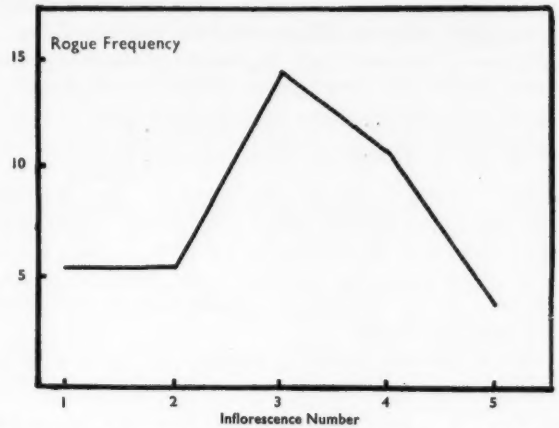


FIG. 4.—Percentage of rogues plotted against the inflorescence number from which the seed was obtained : variety Ailsa Craig : mean of all data from 4 different plants.

5. GENETICAL CONTROL

(1) *Breeding tests*

(a) *Within a high-rogue-producing inbred line.*—Normal and rogue plants selected at random from inbred material of the variety Ailsa Craig were self-pollinated. The seeds were collected from the first four inflorescences, and the seed that was sown was a mixture of equal numbers from the four inflorescences. This obviated any differences which could be due to the inflorescence (table 5).

TABLE 5

Percentage of rogues produced by different rogue and normal plants from an inbred line of Ailsa Craig, germination at 15°, Feb. 1950

	Progeny of selfed rogues			Progeny of selfed normals	
	Total	Rogues		Total	Rogues
R_1	152	18·4 per cent.	N_1	202	10·9 per cent.
R_2	176	14·2 ,,	N_2	379	10·0 ,,
R_3	152	7·9 ,,	N_3	414	7·7 ,,
R_4	167	7·1 ,,	N_4	176	3·9 ,,
R_5	150	4·0 ,,	N_5	161	3·7 ,,
R_6	175	1·7 ,,			
Total	972	8·2 ,,	Total	1332	7·8 ,,

Taken together the percentage of rogues produced from rogue and normal plants is the same. There is, however, considerable variation between plants within both classes. The rogue parents appear to vary more than the normals in this respect. Because of this genetic similarity between rogues and normals no consistent difference would be expected between selfing and inter-crossing them. In the one pair of crosses that was made the results obtained are consistent with this view, as the following figures obtained at 30° show :—

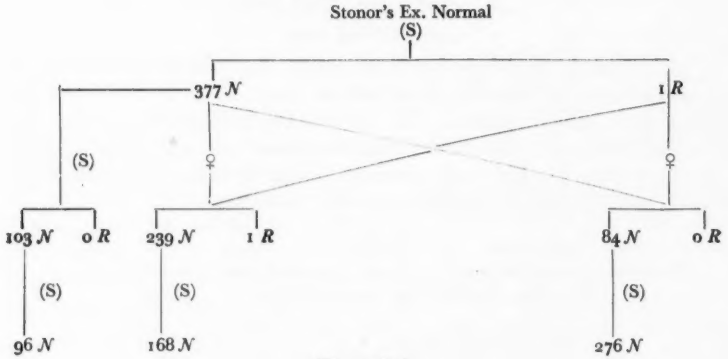
N(13·2)×R(29·1)	18·7 per cent. R
R(29·1)×N(13·2)	11·7 per cent. R

From these results it can be concluded (1) that the difference between the rogue and normal within Ailsa Craig is phenotypic and not genetic. At least, if the difference is genetic, passing through the sex cells has a levelling effect similar to the effect it has on virus-infected plants. (2) All individuals appear to have the potentiality to be either rogue or normal.

(b) *Within a low-rogue producing variety.*—The results within the L.R.P. variety, Stonor's Exhibition, are given in table 6. One rogue

TABLE 6

Progeny obtained from L.R.V., Stonor's Exhibition crosses, germinated at 30° C.



(S) = selfed.

was obtained out of 378 plants which had germinated at 30°. This rogue would produce no seed on selfing : it was more sterile than the rogue in Ailsa Craig. On crossing reciprocally with the normal, one rogue was produced out of 323 plants ; this was about the expected number from normal parents. Selfing for a further generation produced no rogues.

Thus in Stonor's Exhibition which produces about 0·1 per cent. of rogues the difference between rogue and normal, as in Ailsa Craig,

is not inherited : it is phenotypic. The genetic control must thus be sought not within a line but between an H.R.P. and a R.F.* line.

Two hypotheses for these results can be formulated :—

(1) The pure line, Ailsa Craig, is homozygous for a gene or genes with a low penetrance which determine the rogue character. The penetrance being higher at a high temperature, during late development and under low light.

(2) The rogue is determined by competing cytoplasmic particles, the balance of which can be easily changed by the same conditions.

To discriminate between these two hypotheses a series of crosses were made between Ailsa Craig rogue and two different rogue-free lines.

(c) *Within tetraploid rogue line.*—A tetraploid strain of the variety Sunrise was produced by somatic doubling by Mr A. Gavin Brown, who very kindly made the following data available to me. The diploid Sunrise had given under uncontrolled conditions a mean of 5·4 per cent. rogues over a period of years and in the generation prior to chromosome doubling it gave 11·7 per cent.

The tetraploid normal plants produced 37 per cent. rogues and the tetraploid rogues produced 47 per cent. under uncontrolled conditions. The same tetraploid strain germinated at 30° produced 74 per cent. rogues from normal plants.

(d) *Between rogue-producing and rogue-free lines.*—The H.R.P. variety was again Ailsa Craig ; two R.F. lines were used.

1. An inbred line carrying 6 recessive genes as follows :—

d for dwarf-habit	**o** for oval fruits
p for pubescent fruit	**r** for colourless fruit flesh
s for compound inflorescence	**y** for colourless fruit skin

2. An inbred line of *Lycopersicon pimpinellifolium* with yellow fruits. This species is closely related to the tomato, and the cross, the F_1 and later generations are fully fertile.

Neither of these two lines have been known to give a single rogue plant.

Four F_1 families were raised from the **d p s o r y** line ; Ailsa Craig Rogue × *d p s o r y* and reciprocal and Ailsa Craig Normal × *d p s o r y* and reciprocal. The total number of plants raised was 534, out of which no rogues were produced. The four F_2 families derived from the four F_1's totalled 860 plants, none of which was a rogue. All the families were germinated at 30° C. in March. There were, however, three plants which had a slight resemblance to the rogue and were classified as half-rogues. There was some difficulty in scoring in

* R.F. = Rogue-free.

the F_2 generation because of the effects of the many genes segregating, and it may be that the three plants were not half-rogues but contained certain combinations of genes which produce a phenotype resembling the rogue.

The results with *L. pimpinellifolium* in the F_1 and F_2 given in table 7 are similar to those obtained with the *d p s o r y* line. The F_3 takes the analysis a stage further. The results show that the rogue

TABLE 7

Results of F_1, F_2, F_3, B_1 and B_2 generations between Lycopersicon pimpinellifolium (=*P*) *and a rogue from Ailsa Craig* (=*R*). *Germination at 30° C.,* ½*R* = *half-rogue*

F *Generations*						
P×R				R×P		
Total	½R	R		Total	½R	R
148	0	0	F_1	140	0	0
400	0	0	F_2	338	1	0
1120	4	0	F_3 (N)	774	8	0
			F_3 (½R)	173	0	19

Backcross 1 (8 different crosses)

Total	½R	R
1631	1	0

Backcross 2

	Total	½R	R	Total	½R	R	
F_1							
P×P×(R×P)	150	0	0	150	0	10	(R×P)×R×R
P×P×(P×R)	150	0	0	150	0	6	(P×R)×R×R
(R×P)×P×P	150	0	0	150	0	32	R×R×(R×P)
(P×R)×P×P	150	0	0	150	0	41	R×R×(P×R)

is suppressed in the F_1 and F_2 except for one plant in the F_2 which was a half-rogue. This represents 0·13 per cent. of the F_2 generation. The F_3 from this plant gave about 10 per cent. of true rogues. The F_3 from normal F_2 plants contained no true rogues but segregated 0·63 per cent. of half-rogues. Thus we have to conclude that genes or cytoplasm or both from the rogue-free line completely suppress the rogue.

The backcross data given in the same table (7) are more instructive than the F generations. The first generation backcross made to either parent by either of the reciprocal F_1's produces no true rogues. This again is similar to the F_2 generation; and the 0·12 per cent. of half-rogues in the backcross is of the same order as the 0·13 per cent. produced in the F_2 generation. The second generation backcross shows a remarkable change. Backcrossing the F_1 twice to the R.F. parent whether as ♂ or ♀, as expected from the F_2 and 1st backcross results, produces no rogues or half-rogues. Backcrossing twice to the rogue-producing parent, however, gives a high percentage of full rogues. Furthermore there is a significant difference between these backcross families according to whether the rogue is the recurrent female or male parent. The way in which the original cross was made in producing the F_1 generation has no effect on the number of rogues produced in the B_2 generation. The following 2×2 table illustrates these differences.

TABLE 8

Numbers of rogues in B_2 families of 150 plants each, arranged in a 2×2 table to show the effect of the sex of the recurrent R parent and the lack of effect of the direction in which the original cross was made.

Recurrent R parent \ F_1	(R×P)	(P×R)	Total
R ♂	10	6	16
R ♀	32	41	73
Total . . .	42	47	

Thus 4.5 times as many rogues are produced when the recurrent female parent is the H.R.P. line as when the F_1 hybrid and B_1 are the female parents.

The rogues which appear in the B_2 generations were new in one important respect. The rogues in Ailsa Craig can be detected with almost 100 per cent. certainty in the cotyledon stage some 16-22 days after sowing, but the rogues in the B_2 generations could not be detected until much later. No rogues could be detected on the 56th day after sowing and only 4 plants could be classified as half-rogues. On the 66th day rogues could be detected and the number increased to a maximum on the 79th day. Fig. 5 shows this increase in number of rogues recorded at different times after sowing.

The plants finally classified as rogues had, at that time, the full rogue appearance, they were not in any respect half-rogues, despite the late appearance of the rogue characters. They may be called *late-rogues*.

(e) *Selection for rogue and normal within a pure line.*—The material used was Ailsa Craig, selections were made by picking out normal plants for the normal selection line and rogue plants for the rogue

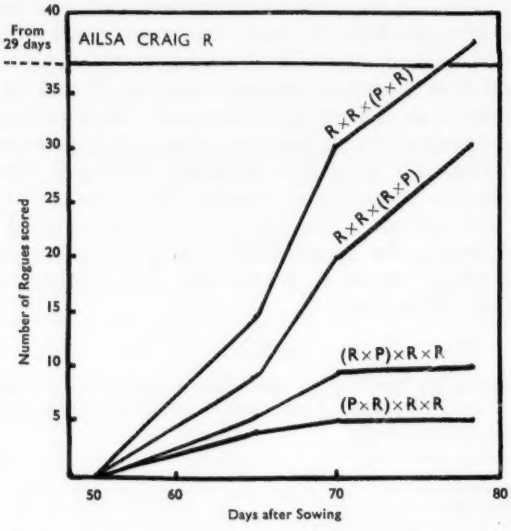


FIG. 5.—Number of rogues produced in the R-line of Ailsa Craig and 4 different B_2 generations of the R-line with R-free line plotted against the number of days after sowing. Note the late development of the R character in the backcrosses as compared with the R-line of Ailsa Craig.

selection line. Two temperatures were used 25-30° and 13°. There were therefore four main selection lines as follows :—

Temperature	*Selection*	
25°-30°	Normal	Rogue
13°	Normal	Rogue

Some of these main lines were split into a series of lines each originating from a different plant. The results of selection are shown in fig. 6. Although the selections were done under different temperatures the rogue assay of each line was done at 30°. Selecting for normal plants was not effective at the high temperature and in fact the percentage of rogues produced increased slightly over three generations of selection. Normal selections at a low temperature showed a slight but non-significant response in the normal direction.

Selection for rogues results in an extremely erratic behaviour from generation to generation with no progress towards increasing the number of rogues.

(ii) *Conclusions*

1. Within an H.R.P. inbred line, rogue and normal plants cannot be differentiated by their breeding behaviour. All plants appear to be potentially rogue.

2. Crossing reciprocally an H.R.P. line with a R.F. line causes suppression of the rogue potentiality in the F_1, F_2, F_3 and B_1. A slight rogue potentiality, as shown by 0·12 per cent. of half-rogues, is manifest in the F_2 and B_1.

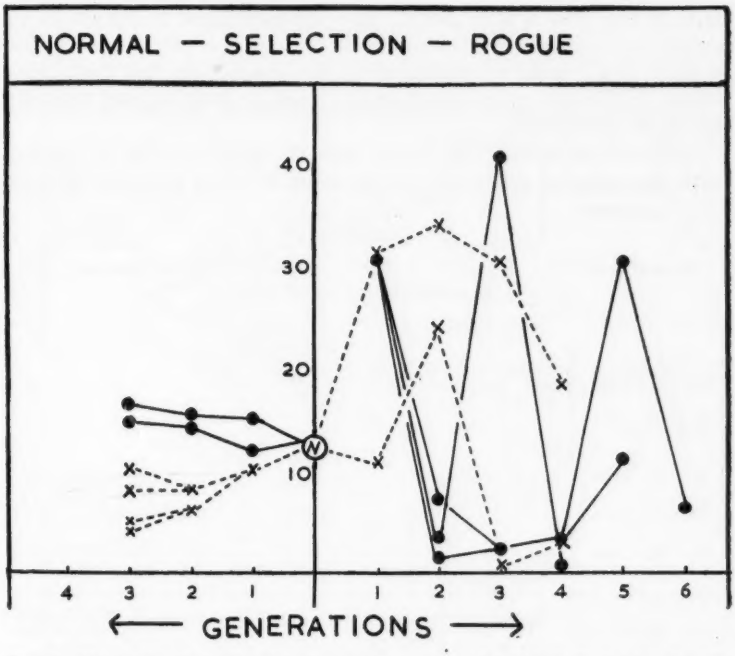


FIG. 6.—The results of selection for rogues and normals under high (30°) and low (12·5°) temperatures. The tests were all made at 30° : variety Ailsa Craig. ——●——, 30° selection : - - - - x - - - -, 12·5° selection. The ordinate represents the percentage of rogues.

The B_2 generation using the R.P. line as the recurrent parent produces true rogues ; and four times as many when the H.R.P. line is female as when it is the male.

3. The rogue plants in the B_2 generation, unlike those from an H.R.P. line, begin as normal seedlings and develop the rogue character later.

4. Selection for normals within an inbred line is not effective except at a low temperature and even then the response is small and of doubtful significance.

6. ALTERNATIVE HYPOTHESES

(i) *One or more genes with low penetrance determine the rogue potentiality of the R line*

This would fit the results obtained within the pure R line in that all plants would be homozygous for the *rr* genes and the phenotypic difference between normal plants and rogues in the line would be due to the difference in penetrance in the different plants.

Would this genic explanation fit the data from intercrossing an H.R.P. line and a R.F. line? The complete lack of rogues in F_1 could be explained by dominance of normal over rogue; complete absence in F_2 could be explained on the assumption that there were about 8 different genes all of which must be homozygous recessive for rogue production.

The critical data of the backcrosses are given in table 9 together with the expected proportion of rogues on different numbers of genes

TABLE 9

Observed results from B_1 and B_2 of R line crossed with R-free line compared with the expectations on a genic basis

Numbers R	Numbers Total	R. Phenotypes	R. Genotypes	Expected on—Genes 2	3	4	5	6	7	8	9
B_1 1*	3262	$\frac{1}{3262}$	$\frac{1}{652{\cdot}4}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$	$\frac{1}{128}$	$\frac{1}{256}$	$\frac{1}{512}$
B_2 89	600	$\frac{1}{6{\cdot}74}$	$\frac{1}{1{\cdot}348}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$	$\frac{1}{128}$	$\frac{1}{256}$

* The actual figures were 1 half-rogues in 1631 plants.

and assuming 20 per cent. penetrance of the bottom recessive. Thus to explain the B_1 generation 9 genes are necessary and to explain the B_2 generation more than 2 genes would give a significantly different result from the one observed.

Secondly the B_2 results were obtained from 4 different B_2 plants and no difference in results in the B_2 could be attributable to differences between the B_1 plants within a family, which must occur on a genic explanation. This fact, together with the large number of genes invoked in B_1 precludes a possible explanation of the B_2 results on an inadequate sample of the B_1.

Thirdly on the evidence from temperature effects, selection results, and reciprocal backcross differences, a purely genic hypothesis must be excluded.

(ii) *The difference between rogues, half-rogues and normals within an H.R.P line is due to a re-orientation of an unstable equilibrium in the somatic tissue during differentiation and development.*

(*a*) This re-orientation does not affect the germ cells.

(*b*) Temperature, light and genotype influence the re-orientation of the unstable equilibrium.

(*c*) The direct control of the rogue-normal re-orientation is by a cytoplasmic factor which is transmitted more efficiently by the eggs than by the pollen.

How does this scheme fit the facts ?

The difference between rogues and normals within an inbred H.R.P. line has been shown to be phenotypic and is not inherited. Selection for rogues and normals is ineffective. The effect of environ-

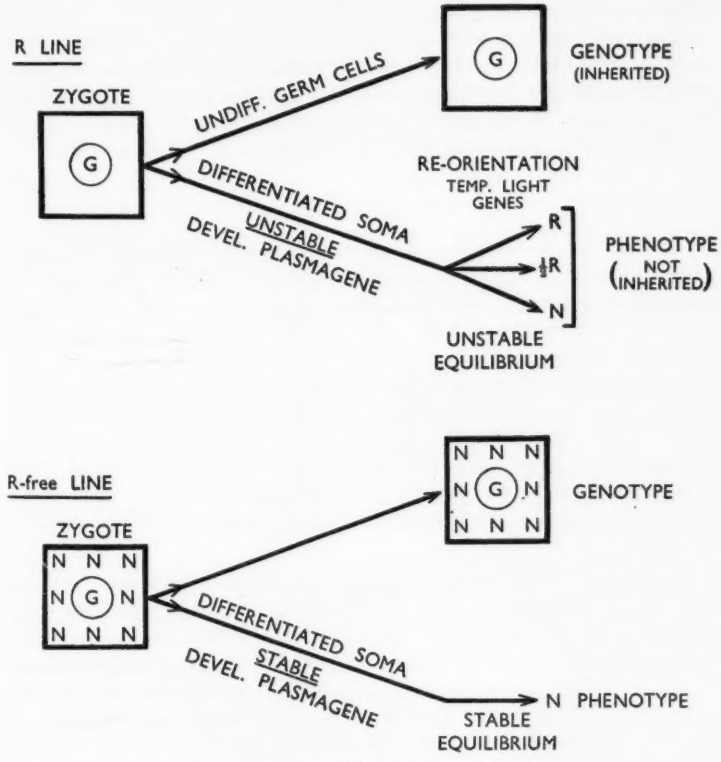


FIG. 7.

ment is well defined : it re-orientates a seed along one of three paths, normal, half-rogue and rogue. This aspect of the problem is clear and accords with the hypothesis.

The genetical control of the unstable equilibrium, which allows re-orientation to occur, has not been fully clarified. A purely genic basis on any number of genes with or without modification is incompatible with the data. The alternative to a direct genic control is a cytoplasmic determinant. But the difficulty in assessing a cytoplasmic factor in the present problem is the lack of big reciprocal differences in transmission. There are, however, reciprocal differences in the B_2 generations which show that the genetical determinants of the normal (stable equilibrium) are transmitted four times more efficiently through the eggs than through the pollen. On the other hand there are no reciprocal differences which can be attributed to the F_1, F_2 and B_1 generations. To reconcile these differences an added complication must be introduced : the reciprocal difference in transmission only operates when the number of determinants falls below a certain threshold value.

The differences in transmission between different inflorescences is to be expected with a cytoplasmic determinant, and despite the great difficulty with the present problem of separating genes and cytoplasm the cytoplasmic hypothesis is the only plausible one.

The tentative scheme is illustrated in fig. 7.

7. DISCUSSION

In the past the idea had become firmly established that the main criterion for a cytoplasmic determinant in a higher plant was its maternal inheritance. Without this, other evidence was of no value. Recently, Darlington (1949) pointed out, however, that the best examples of cytoplasmic determinants—the chloroplasts—in the classical work of Baur and Chittenden were biparentally inherited. He also drew the important conclusion from the work of Bateson and Pellew (1920) not only that the rogue-pea was due to a cytoplasmic difference but that the rogue plasmagenes were transmitted more frequently through the pollen than through the egg. Biparental transmission of a plasmagene has been explained by Darlington in two ways :—

(1) that when the plasmagene reproduction is faster than cell reproduction then the plasmagene will accumulate most in the slowest growing cell of the plants—the pollen grain.

(2) That if the abnormal plasmagene suppresses the normal then the one or two plasmagenes which come through the pollen will eventually suppress the large number of normal plasmagenes in the egg.

If we turn to microorganisms maternal inheritance has not been the stumbling block to the interpretation of cytoplasmic inheritance that it has been in the flowering plants, because generally the two fusing cells are of equal size. In yeast, Ephrussi *et. al* (1949) and Chen,

Ephrussi and Hottinguer (1950) have interpreted their results with the "petite" type which arises spontaneously and after acriflavine treatment during vegetative budding, on a cytoplasmic factor. Their data on a mendelian interpretation would require 6-20 nuclear genes. The present results on the rogue tomato do not show marked reciprocal differences except in certain families. The results cannot be explained on Mendelian lines however many genes are invoked as we saw.

The rogue tomato is similar in many respects to the rogue pea and the bolter potato. It differs, however, from the rogue pea in that the rogue is suppressive to normal in the pea while in the tomato the reverse is true—the normal suppresses the rogue. The bolter potato (Stanton, 1952) which is an abnormal type arising vegetatively, bears the greatest resemblance to the rogue tomato in that "the frequency of bolting in the progeny depends more on the variety than on the state, bolter or normal of the parents." The bolter-normal difference within a variety of potato, as the rogue tomato, is phenotypic and not genetic. The rogue-normal difference in peas, on the other hand is genetic as well as phenotypic.

The complexity of systems of competing cytoplasmic determinants is evident from all work on cytoplasmic inheritance. This is very clearly shown by the work of Beale (1952) with the antigenic types in *Paramecium* where the system lends itself to a precise analysis of the interaction of genes, cytoplasm, and environment.

The induction of a stable and distinct class, the half-rogues by short heat treatments is evidence that different equilibria between the competing plasmagenes can be attained in the cells. This would appear to be a general feature, for intermediate and transforming types are also found in the rogue pea and the bolter potato. The half-rogues also appear to have their counterpart in the "resistant non-killers" in *Paramecium* which are intermediate between "killer" and "sensitive", Sonneborn (1946). These were produced by short heat treatments as were the half-rogues in tomatoes; they differ, however, in that the "resistant non-killers" change to "killers" while the half rogues are stable.

The effect of temperature during germination on the rogue is very similar to that found by Sonneborn for the killer and by Beale (*loc. cit.*) for antigens in *Paramecium*; to the effect on CO_2 sensitivity in *Drosophila melanogaster* (l'Héritier, 1948); and on the "Sex ratio" phenomenon in *Drosophila bifasciata*, Magni (1952).

The origin of the rogue tomato is obscure; it may arise by mutation. Evidence from commercial growers points to this possibility. For example, the variety Stonor's Exhibition which has given only 0·1 per cent. of rogues at the John Innes Horticultural Institution has been known to produce in some commercial stocks as much as 30 per cent. of rogues.

The problems which require solving are : the origin of the plasmagene differences, the precise nature of the competition between plasmagenes, and the nature of the intermediate types. The solution to these problems may have to be sought in more favourable material —material in which the actual plasmagenes and their differences can be seen in the cell.

Acknowledgments.—I wish to thank Prof. C. D. Darlington for valuable help in discussing the problem during the course of the work ; Miss V. P. J. Clarke for making many of the crosses and Miss L. K. Crowe for the diagrams.

8. SUMMARY

1. In several pure lines of tomatoes an abnormal but stable growth type of plant—*rogue*—occurs in proportions ranging from 0·1 to 20 per cent. according to the line.

2. Four factors increase the frequency of rogues. (1) A high temperature during germination increases the frequency of rogues from 1·4 per cent. at 13° to 13 per cent. at 25°-30°. This is due to a transformation or re-orientation in the seed. Intermediate types—*half-rogues* are produced by short heat treatments. (2) Low light intensity and short days during germination. (3) The position on the plant of the inflorescence from which the seed is obtained. The 1st and 2nd inflorescences give a lower percentage of rogues than the 3rd and 4th. (4) The genetic constitution.

3. Rogues and normals within a pure line behave similarly on selfing : both give approximately the same percentage of rogues, although rogues vary more. The difference between rogue and normal therefore is phenotypic and not genetic.

4. Crossing a rogue onto a rogue-free line results in suppression of the rogue in the F_1, F_2, F_3 and B_1 generations. The B_2 generation to the R line has its rogue potentiality restored. Reciprocal differences in transmission were only found in the B_2, where the female was four times as efficient in " normal " transmission as the male.

5. The results are inexplicable on any nuclear genic hypothesis because non-segregation in the B_1 demands at least 9 genes while segregation in the B_2 does not allow of more than 2 genes.

6. A hypothesis based upon a " normal " plasmagene in the R-free line and its absence in the R line is discussed.

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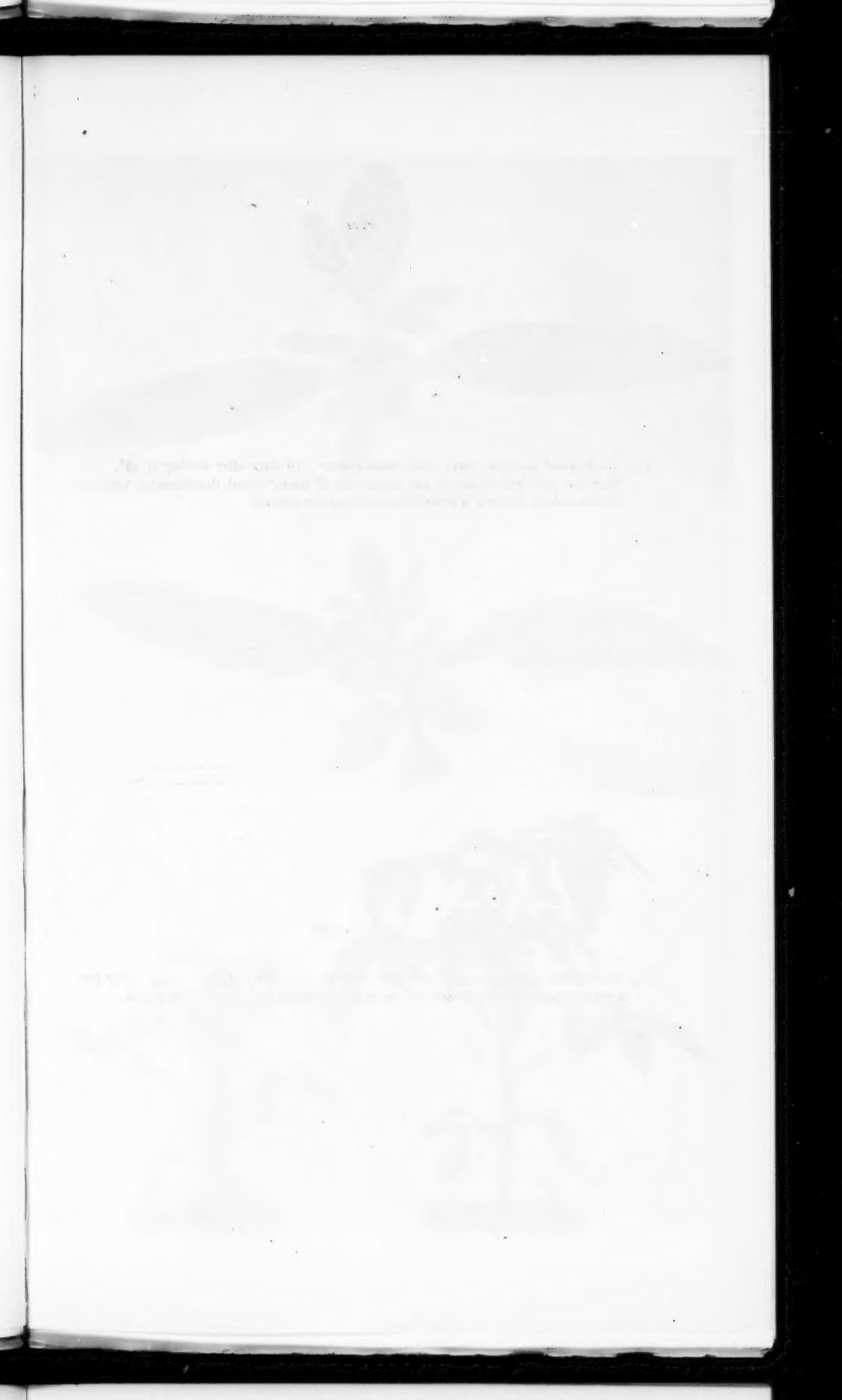

Plate

FIG. 1.—Normal seedling (top), and rogue (below), 16 days after sowing at 26°. ×2. Note the two true leaves in the rogue are of nearly equal development, while in the normal the first leaf is more advanced than the second.

FIG. 2.—Normal seedling (right) and rogue (left) 56 days after sowing. ×0·4. Note the short internodes, smaller leaves and development of the side shoots in the rogue.

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AN ANALYSIS OF THE ABO BLOOD-GROUP RECORDS OF THE NORTH OF ENGLAND

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1. INTRODUCTION

THE very extensive records of the National Blood Transfusion Service have been used for a number of surveys in various parts of the British Isles. These surveys have varied greatly in scale and in the amount of detail included. Only a relatively small part, however, of the information available has actually been utilised and it seemed desirable, as a matter of some urgency, (i) to investigate systematically what use could be made of the present accumulation of particulars of donors' groupings, (ii) to search for possible difficulties and sources of error that might make their use dangerous, (iii) to discover economical ways of carrying out the analyses, and (iv) to suggest additional items of information of potential value that might be incorporated without too much practical difficulty. Apart from these questions of technique, it is desirable to discover how fine an analysis is likely to prove profitable. Should it be found, for example, that there is little variation in gene frequencies within large urban areas, then relatively modest samples would suffice. If, on the other hand, such areas showed real variations, then it would be worth while carrying out much more detailed counts. The broad outlines of the variation, particularly of genes O and A, are known. But, underlying these, are there smaller local variations from place to place? This was not known. With these considerations in mind, Dr C. D. Darlington took the initiative in calling together a group of interested workers consisting of Dr W. d'A. Maycock, Dr A. E. Mourant, Dr R. R. Race and myself, and the Nuffield Foundation generously provided a grant which enabled the work to be carried out.

The main project, it was decided, should be a pilot survey, carried out in one of the Regions of the National Blood Transfusion Service. The Region selected was that based upon Newcastle-upon-Tyne and comprising the counties of Northumberland, Durham, Cumberland and Westmorland, with the northern part of the North Riding of Yorkshire. The authorities of the Region very kindly offered to make the cards available in batches for copying and have taken

throughout a great amount of trouble in helping with the complicated arrangements that proved necessary. It was known that the records of this Region were likely to be complete, in particular that the cards of resigned donors removed from the panel had been maintained since the beginning of the Service. Some previous surveys, for example my own in south-western England (1948), have included records of donors who had been provisionally grouped and added to the panel at the time of registration. This procedure makes a certain proportion of grouping errors inevitable and it is by no means certain that the errors will be independent of the location of the centres at which the preliminary grouping is carried out. Hence fine geographical sub-division, or a comparison of rural versus urban areas, are both unsafe. In the present material, donors were not added to the panel until they had made the first donation of blood, so that grouping errors were minimised and such as may remain are likely to be quite independent of geographical location. This makes it possible to sub-divide the material in any way desired.

2. MATERIAL AND TECHNIQUE

(i) *General*

The record comprised cards, one for each donor, there being, as is usual, no duplicates. Hence it was arranged that batches of cards should be sent to the London School of Hygiene and Tropical Medicine as they could be spared. The relevant particulars for each donor were copied on to a Powers-Samas punched card. When each batch was received the first step was to stamp a serial number on each original card, the serial number being subsequently copied on the corresponding punched card. This ensured that all cards were duly received and eliminated the risk that cards might be sent in twice. It also made it possible to resort the original cards before returning them into the order in which they were received.

A number of panels, maintained separately, referred to bodies of non-local donors, chiefly those at large Service establishments. These donors were not included in the count. One special panel was that of the Ministry of National Insurance ; these donors were included and a special indication was punched on the Powers card.

With one exception, the area code number, which is dealt with in the next section, the particulars to be copied were so simple that coding slips were unnecessary, the punched cards being prepared directly from the originals. The particulars copied, apart from area, were as follows :—

1. Serial number, as already mentioned.
2. Live or resigned panel.

3. Family name, which was punched in full.
4. Sex and marital status, under the headings : man ; married woman ; single woman ; woman, marital status not recorded; sex not recorded.
5. ABO blood-group.
6. Rhesus positive or negative (when recorded).

The ages of donors were seldom recorded and so are not dealt with in this paper.

(ii) *Classification by area*

This was much the most difficult and time-consuming part of the task. The panels in which the original cards were maintained were much too large to be used. Moreover, for the practical purpose of bleeding donors often attend a centre near their place of work rather than their home, which, it had been decided, must be the basis of classification. Accordingly, every card had to be examined for home address.

The first principle in making the classification by area was to make the sub-division as fine as possible. Minimum size in number of donors was determined by the comparisons to be made. These were :—

1. The ratio A/(O+A), originally used by Fisher and Taylor (1940). The variations in the proportions of genes O and A are largely reciprocal, so this comparison is a particularly useful one.
2. The ratio (B+AB)/(O+A+B+AB), which is sufficient for an analysis of fluctuations in the frequency of gene B.

It will be realised that with a very large number of areas to analyse and compare the convenience of examining heterogeneity by means of two simple $2 \times N$ comparisons is very great. For the first comparison the minimum number of donors needed in each area is about 20 ; for the second about 70. So 20 was fixed as the minimum number ; for making the second comparison the smaller areas would then have to be added together as was necessary in order to secure a minimum of 70.

The next principle adopted was to keep separate as far as possible individual towns, parts of towns and villages. The largest city, Newcastle-upon-Tyne, was already divided into 4 separate panels ; Gateshead, south of the river, providing a fifth. Where a definite district in a large city or town could be identified by a name in the address this was kept separate. In consequence certain larger towns and particularly the divisions of Newcastle tended to be to some extent residual areas left after identifiable districts within them or adjoining them had been counted separately. The addition of towns

and villages to rural areas was avoided as far as possible ; instead rural areas were, when it was practicable, added to each other. In forming the geographically larger rural and semi-rural areas, attention was paid to the features, such as valleys, roads and railways, that might be expected to facilitate communication.

When a batch of cards was received and the serial numbers had been stamped it was sorted as finely as possible by home address, the places being identified with the help of a set of one-inch Ordnance Survey Maps and Bartholomew's Gazetteer of the British Isles. A card index of every named locality, with map references, was built up as the work proceeded and proved very useful. The batches of cards were received, as they could be spared, more or less indiscriminately from centres all over the area. Panels overlap, and, furthermore, live and resigned panels were usually received separately. Thus at no stage could the material be reviewed as a whole and at times a good deal of guessing had to be practised. Area numbers had to be allotted and punched for considerably fewer than 20 donors in the anticipation that subsequent batches would make up the number. In individual instances single cards would be received from areas not yet dealt with (usually referring to donors working relatively far away from their homes) ; such cards were punched omitting the area code number and a slip was attached giving the full address, the punched card being then kept in a deferred file until cards were received from the area concerned. Not unnaturally, when the count was finished, a number of areas were found not to have attained the minimum number ; these were added to each other or to adjacent areas as proved most convenient. On the other hand, the guess was sometimes a poor one in that a single code number was allotted to an unnecessarily large area, which, it was subsequently found, could have been sub-divided. Nor was it found when the work was completed that the boundaries of some of the scattered areas had been happily drawn.

A few donors lived over the Border in Scotland ; they were omitted from the count. Similarly, a very few donors lived south of the southern boundary of the Region and they too have been omitted.

When the copying was finished and the cards were counted, it was found that the total number of donors was 54,579, divided into 321 areas, each containing at least 20. The largest single area comprised 3260. Of the 321 areas, 197, comprising all but some 8000 donors, were single towns, parts of towns, or villages ; the remaining 124 areas covered more than single towns or villages ; sometimes indeed, in the more sparsely populated rural areas, considerable tracts of country. It will be seen, therefore, that the intention of keeping towns and villages separate as far as possible was largely realised, when considered in terms of donors.

(iii) *The progressive addition of the areas into larger aggregates*

As already mentioned, while the 321 original separate coded areas were large anough for examining fluctuations in the ratio A/(O+A), many fell short of the minimum number of 70 required for the investigation of fluctuations in the B ratio and so areas containing less than this number had to be added together. The principles observed in doing this and in all subsequent additions were the same as before, namely, to keep single towns, parts of towns and villages separate as far as possible and to follow natural and other features in adding other areas. Everything was done by inspection of the one-inch maps (on which the areas had been marked out). Needless to say, neither at this stage, nor at any other, was any regard paid to the proportions of donors belonging to the various groups, the additions being determined solely by geographical considerations. Naturally, the adding did not always prove as convenient as if the areas had originally been selected with the number 70 in mind. For example, it would happen that the only area to which a very small area could be added already contained more than 70 donors; or three areas all just under 70 would have to be combined. When the additions had all been made it was found that the number of areas at this second stage had been reduced to 196.

An observation made at this stage was helpful in determining further procedure. The ratio A/(O+A) was highly heterogeneous as between the 321 areas. When the additions had been made to give the 196 areas, it was noted that all the significant A : O heterogeneity lay between the 196 areas and none within them. This suggested that the process of addition should be continued. There was now no factor in the analysis which determined any particular minimum size, so the figure of 300 donors was arbitrarily chosen as one that would roughly halve the number of areas. The number of areas was now reduced to 90. Once again it was found that all the significant A : O heterogeneity lay between the 90 areas. The effect on the ratio (B+AB)/(O+A+B+AB) could also be investigated at this stage. At the original (for the B comparison) stage of 196 areas the ratio was highly heterogeneous; but just as with the O : A ratio, all this heterogeneity lay between the 90 aggregates of minimum number 300.

Still further addition was indicated. The minimum size had to be 600, as it was clear that at the stage now reached the areas of 300 could usually only be reasonably added in pairs. The number of areas was now reduced to 52 and once again, for both comparisons, all the significant heterogeneity lay between the 52 areas. As before, addition in pairs had to be the basis of the next step, the new total being 29 areas with a minimum of 1200 donors in each. It was now found that the process of addition had been carried too far. In respect

of both ratios, the reduction to 29 areas left significant heterogeneity within those areas.

The results are shown in table 1 and are quite unequivocal. Starting with very small areas, in terms of numbers of donors, it is possible to go on adding adjacent areas, purely geographically, up to minimum aggregates of 600 donors. The aggregates are homogeneous within themselves and all the heterogeneity, both of the

TABLE 1

Effect on heterogeneity of progressive addition of the 321 areas into larger aggregates

Minimum donors	D. of F.	D. of F. diffs.	A/(O+A)			(B+AB)/(O+A+B+AB)		
			χ^2	χ^2 diffs.	P	χ^2	χ^2 diffs.	P
20	320		*388·63*			...		
		125		136·22	0·23		...	...
70	195		*252·41*			*262·18*		
		106		93·97	0·79		112·98	0·31
300	89		*158·44*			*149·20*		
		38		40·38	0·37		37·51	0·50
600	51		*118·06*			*111·69*		
		23		35·83	0·043		40·55	0·013
1200	28		*82·23*			*71·14*		

Summary

Variation	A/(O+A)			(B+AB)/(O+A+B+AB)		
	D. of F.	χ^2	P	D. of F.	χ^2	P
Between 52 areas . .	51	*118·06*	<0·0001	51	*111·69*	<0·0001
Within 52 areas . .	269	*270·57*	0·46	144	*150·49*	0·34

Note.—As the results are so clear it is unnecessary to recalculate the values of χ^2 using adjusted divisors, *pq*, and divisors derived from the totals are used throughout.

ratio A/(O+A) and of (B+AB)/(O+A+B+AB), lies between them. But the process cannot be carried further. Areas with a minimum number of donors of 1200 are significantly heterogeneous within themselves in respect of both ratios.

Rough estimates may be attempted of what these numbers of donors mean in terms of total population. Taking the four counties and the northern part of the North Riding as a whole, and quoting figures to the nearest thousand throughout, the Registrar-General's estimate of total population was 2,834,000 in 1938 and 2,836,000 in

1947, giving a mean of 2,835,000. Dividing this figure by 52, the number of areas that can reasonably be formed by progressive addition, the result is 55,000 ; division by 29, the number of the larger areas that cannot be so formed, gives 98,000. Alternatively, one can estimate the total population corresponding to the minimum number of donors, that is, 600 and 1200. The result is 31,000 and 62,000 respectively. As in any transfusion service, the region is covered with very variable degrees of thoroughness, the main factor being that it is economical to concentrate on the larger centres of population. Bearing this weighting in mind, it may be concluded that smaller areas can be progressively added, without introducing heterogeneity within themselves up to a point which corresponds very roughly to a total population size of the order of 50,000.

(iv) *The gene frequencies and Fisher's test for group proportions in the* 52 *areas*

The basic data are presented in table 2. As others with particular anthropological, historical or geographical hypotheses to test might like to have more detail and so be enabled to add areas differently, the totals and the group percentages (from which the original numbers can be reconstructed) are shown separately for the penultimate stage of 90 areas.

The gene frequencies and the χ^2 for Fisher's test for reasonableness of group proportions (Dobson and Ikin, 1946) are shown for the larger aggregates, comprising 52 areas. The results of the test can only be considered very satisfactory. All large bodies of data show a deficiency of group AB ; and the amount of the deficiency seems to be related to the care with which the grouping has been done and clerking errors avoided. Doubtless some at least of the larger deficiencies shown in some samples is to be attributed to such errors as some A_2B donors being wrongly assigned to group B. But even with the most careful precautions, there are apparently always, or almost always, too few AB's. Thus in Dobson and Ikin's enormous and very accurately grouped sample of 190,000 the deficiency is 84, which does not, however, attain the level of significance. It is satisfactory that in the present relatively large sample, the deficiency in the total is no more than 87, giving a non-significant χ^2. The χ^2's for the 52 individual areas are even more satisfactory. There is one highly significant figure of 7·6 and one significant figure of 4·7, which seems very reasonable with 52 observations. In fact, the 52 χ^2's have a distribution which is remarkably close to theoretical expectation. As far, then, as one test (with a single degree of freedom) can be taken to indicate a satisfactory body of data, the records of this northern region of England give no indication of anything suspicious.

TABLE 2

Four northern counties of England, with part of North Riding of Yorkshire. Numbers and group frequencies for 90 areas, with gene frequencies and Fisher's χ^2 for the 52 larger aggregates.

	No.	Group frequencies per cent.				Gene frequencies per cent.			AB Obs.-Exp.	χ^2
		O	A	B	AB	O	A	B		
1 Berwick-on-Tweed, Ellingham	354	52·82	35·59	9·89	1·69	72·4	21·5	6·1	−5·36	0·99
2 Alnwick, Netherwhitton	460	52·83	36·30	8·70	2·17					
3 *Ashington*	608	53·13	36·68	8·22	1·97	72·7	21·8	5·4	−2·47	0·31
4 Newbiggin, Widdrington	383	45·69	42·82	9·92	1·57	67·7	24·8	7·6	−10·34	2·82
5 *Morpeth*	308	47·40	37·01	12·34	3·25					
6 Stannington, Pegswood	506	49·80	36·76	9·29	4·15	73·5	20·2	6·4	+6·95	1·72
7 Seaton Delaval, Bebside	320	59·38	28·13	10·31	2·19					
8 *Blyth*	495	52·32	36·36	7·47	3·84	72·3	22·0	5·7	+2·13	0·17
9 Seaton Sluice, Shire Moor	302	51·99	36·75	10·26	0·99					
10 *Monkseaton*	432	46·99	40·97	9·49	2·55	70·1	24·2	5·7	−6·25	1·26
12 Cullercoats, New York	368	52·45	39·13	7·07	1·36					
11 *Whitley Bay*	683	51·10	39·09	8·20	1·61	71·1	23·4	5·5	−6·71	0·84
13 Tynemouth	347	52·45	39·77	5·48	2·31	71·9	23·0	5·1	−1·14	0·06
15 *Willington Quay-upon-Tyne*	303	51·16	36·96	9·90	1·98					
14 *North Shields*	1535	52·05	37·92	7·56	2·48	72·2	22·7	5·1	+2·73	0·16
16 Longbenton, Wide Open	349	49·86	36·96	10·60	2·58	69·7	23·3	7·0	−10·72	2·96
17 *High Heaton, Forrest Hall*	494	48·79	39·47	10·12	1·62					
18 *Wallsend*	703	50·36	36·13	11·52	1·99	70·5	21·9	7·7	−9·86	2·94
19 *St Anthony's, Walkerville*	353	49·86	39·94	8·22	1·98					
20 *Walker*	408	53·19	38·48	6·62	1·72	71·6	23·0	5·4	−5·60	0·86
25 *Byker*	304	51·32	36·51	9·87	2·30					
21 *Heaton, North Heaton*	678	47·35	41·59	7·52	3·54	69·1	25·6	5·3	+5·80	1·30
22 *Newcastle East*	933	46·41	37·62	12·33	3·64	68·0	23·5	8·5	−3·40	0·22
23 *Gosforth*	624	50·00	37·66	9·78	2·56	69·2	23·0	7·8	−12·56	3·06
24 *Newcastle North*	383	45·95	37·60	14·36	2·09					
26 *Newcastle Central*	2014	46·33	39·67	11·52	2·48	67·6	24·5	7·9	−29·44	7·58
27 Westerhope, Coxlodge	409	48·90	38·14	9·05	3·91	71·2	23·6	5·2	+11·11	4·73
28 *Fenham*	377	51·19	39·26	5·84	3·71					
29 *Newcastle West*	2230	47·94	37·62	11·35	3·09	69·1	23·2	7·7	−11·49	1·16
30 *Denton, Elswick*	386	48·70	40·16	8·29	2·85	70·1	24·7	5·2	+5·27	1·01
31 *Benwell, Scotswood*	385	48·83	40·78	6·75	3·64					
32 Wylam, Lemington-on Tyne	338	55·03	31·95	10·95	2·07	71·2	22·3	6·5	−4·46	0·74
43 Winlaton, Ryton	330	46·97	42·12	8·48	2·42					
33 *South Shields*	1830	48·63	38·96	9·29	3·11	69·8	23·9	6·4	+1·39	0·02
34 Cleadon, Harton	374	51·60	35·29	9·09	4·01	72·1	21·8	6·1	+0·32	0·00
35 *Jarrow*	363	52·34	36·91	9·37	1·38					
36 *Hebburn*	480	49·79	38·75	7·92	3·54	72·0	22·7	5·3	+9·17	2·70
37 *Felling, Heworth*	487	52·98	36·34	7·60	3·08					
38 *Gateshead*	2101	49·21	39·08	8·66	3·05	70·2	23·8	5·9	+4·73	0·27
39 *Dunston*	321	48·60	37·38	9·35	4·67	67·5	26·1	6·4	+1·74	0·08
42 *Blaydon, Swallwell*	469	43·28	45·20	8·74	2·77					
40 Chester-le-Street, Birtley	679	48·01	35·79	11·63	4·57	69·7	22·4	8·0	+7·01	1·46
41 Low Fell, Whickham	645	50·54	38·45	9·15	1·86	70·7	23·1	6·1	−6·48	1·64
44 Prudhoe, Greenside	456	53·29	34·43	8·77	3·51	72·7	21·9	5·4	+3·57	0·49
54 Rowlands Gill, Medomsley	370	51·89	38·92	7·30	1·89					
45 *Southwick, Roker*	574	45·47	45·12	6·62	2·79	66·3	28·1	5·6	−5·36	0·66
47 Fulwell, Whitburn	354	42·09	46·05	9·60	2·26					
46 Boldon Colliery, Pallion	391	51·15	38·11	8·44	2·30	71·1	23·6	5·3	+1·70	0·12
49 Ford, Humbledon	305	49·51	40·33	6·89	3·28					

TABLE 2 (*continued*)

	No.	Group frequencies per cent.				Gene frequencies per cent.			AB Obs.-Exp.	χ^2
		O	A	B	AB	O	A	B		
48 *Sunderland*	2162	49·63	39·13	8·74	2·50	70·3	23·7	5·9	−7·17	0·60
50 Silksworth, Grindon	344	52·03	39·24	6·98	1·74	72·8	21·5	5·7	−3·18	0·41
51 Seaham, Ryhope	396	54·29	33·33	10·10	2·27					
52 Hetton-le-Hole, Deneside	412	48·06	41·75	7·04	3·16	70·5	24·5	5·0	+1·91	0·14
53 Stanley, Pelton	368	51·36	38·86	7·61	2·17					
55 Shotley Bridge, Blackhill	413	48·43	39·23	9·20	3·15	71·3	22·9	5·8	+0·57	0·01
56 *Consett*	314	53·82	35·99	7·96	2·23					
57 Durham, Houghton-le-Spring	368	46·47	40·22	10·33	2·99	69·4	24·5	6·1	−2·28	0·18
60 Crook, Spennymoor	305	50·49	40·00	7·21	2·30					
58 Sedgefield, Thornley	373	47·72	38·07	9·65	4·56	69·8	23·5	6·7	+7·83	1·92
59 *West Hartlepool*	358	48·88	37·99	9·78	3·35					
61 Bishop Auckland, Shildon	325	45·85	44·00	8·31	1·85	69·3	25·4	5·3	−4·98	1·03
62 Willington, Barnard Castle	305	51·15	40·00	6·89	1·97					
63 Kirkby Stephen, Appleby	549	46·45	41·89	8·74	2·91	68·6	25·4	6·1	+3·09	0·25
87 Penrith	330	47·58	39·70	8·48	4·24					
64 *Darlington*	2336	48·42	39·04	9·55	3·00	69·7	23·9	6·5	−3·34	0·09
65 Richmond, Stillington	555	49·37	38·74	9·01	2·88					
66 Saltburn, Nunthorpe	349	44·70	42·98	10·03	2·29	69·0	24·8	6·1	−0·40	0·00
67 *Redcar, Dormanstown*	387	50·39	38·24	7·75	3·62					
68 Grangetown, Stainton	503	47·32	41·15	8·55	2·98	68·1	25·0	6·9	−5·89	0·90
69 *South Bank*	275*	45·82	39·64	12·36	2·18					
70 *North Ormesby*	614	47·88	40·55	8·96	2·61	69·0	24·8	6·2	−2·90	0·31
71 *Middlesborough*	2399	46·98	42·31	7·84	2·88	68·5	25·8	5·7	+0·00	0·01
72 *Linthorpe*	362	46·69	40·06	9·94	3·31					
73 *Billingham*	349	49·28	39·26	7·74	3·72	68·5	25·4	6·1	+6·81	1·29
74 *Norton*	466	44·42	42·06	9·44	4·08					
75 Thornaby, Hartburn	682	48·97	37·10	10·26	3·67	70·2	22·9	7·0	+3·28	0·36
76 *Stockton-on-Tees*	1005	47·56	42·09	8·06	2·29	68·8	25·6	5·6	−6·03	0·88
77 Hexham, Stocksfield	336	51·49	35·71	10·12	2·68	72·4	22·1	5·5	+2·40	0·27
78 Haydon Bridge, Bellingham	306	52·94	37·91	6·21	2·94					
79 Longtown, Brampton	302	54·30	31·13	10·60	3·97	72·9	20·1	7·1	+4·13	0·71
80 Harraby, Scotby	333	51·35	34·83	10·81	3·00					
81 *Stanwix*	353	58·07	30·03	9·92	1·98	72·7	21·1	6·3	−9·80	0·74
82 *Carlisle*	3260	52·39	35·67	9·51	2·42					
83 Silloth, Workington	601	51·75	38·27	7·32	2·66	72·7	22·5	4·9	+6·26	1·00
84 *Maryport*	349	53·58	36·68	7·45	2·29					
85 Whitehaven, Cleator Moor	371	52·83	37·20	7·01	2·96					
86 Keswick, Ambleside	386	47·67	40·93	9·07	2·33	68·6	24·5	6·9	−5·47	0·90
88 Windermere, Coniston	307	47·23	38·76	11·07	2·93					
89 *Kendal*	395	50·89	37·22	10·13	1·77	70·7	23·2	6·1	−3·74	0·46
90 Kirkby Lonsdale, Arneside	375	49·60	39·73	7·73	2·93					
Total	54,579	O, 49·484 ; A, 38·627 ; B, 9·126 ; AB, 2·763				70·3	23·5	6·2	−87·13	3·41

Notes.—1. Numbers in groups can be reconstructed from totals and percentages.

2. Names in italics denote areas composed of single towns, parts of towns, or closely adjacent urban localities.

3. When possible double names indicate extent of area.

* A mistake was made in keeping South Bank separate at the 300 donor stage. Rearrangement of the areas would have been troublesome and was not attempted.

3. GEOGRAPHICAL VARIATIONS

(i) *Frequencies of genes O and A*

The maps show the frequencies of the three genes, giving each of the 52 areas separately. It will be realised that the approach to this pilot investigation has been empirical. The main purpose of the survey was to test the nature and reliability of the recorded data and to see what relatively fine fluctuations may exist that the records of the National Blood Transfusion Service can be expected to elucidate and which it might be profitable to study in other areas in the future. Moreover, such analysis as has been carried out so far on this material using the family names gives the hope that further useful information will be forthcoming. This will be dealt with in another paper. For the moment, it seems best to look at the figures as they stand, simply in terms of the 52 areas, without attempting to make alternative additions, and see what deductions can be drawn. More detailed analysis in the light of the history and geography of the area can follow later.

Inspection of the O and A maps strongly suggests that much the most important feature in the variability of the frequencies of these genes is the south-north gradient in A and O frequencies. A number of experiments were tried and it soon became clear that it is possible to draw a single line from east to west, dividing the whole region into two in such a way that the two areas are homogeneous within themselves and all the significant heterogeneity lies between them, as shown in table 3.

TABLE 3

Analysis of the heterogeneity of the 52 areas in respect of the variability of O and A. Division of region into two, as indicated on O map

	No. of donors	No. of areas	O/(O+A) per cent.
Northern . . .	19,460	20	41·43
Southern . . .	35,119	32	45·19
Total . .	54,579	52	...

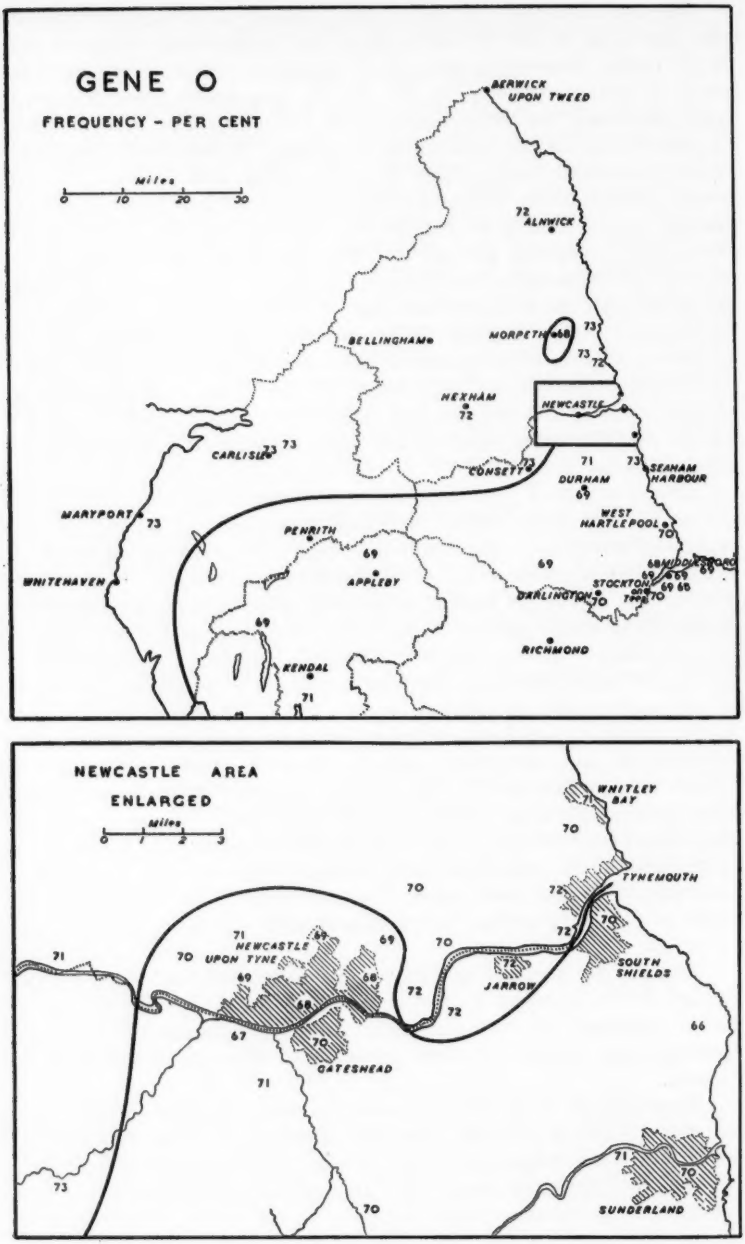
Variation	D. of F.	χ^2	P
Within northern areas . . .	19	*16·19*	0·64
Within southern areas . . .	31	*38·43*	0·17
Between the two areas . . .	1	*63·44*	<0·0001
Between all areas	51	*118·06*	<0·0001

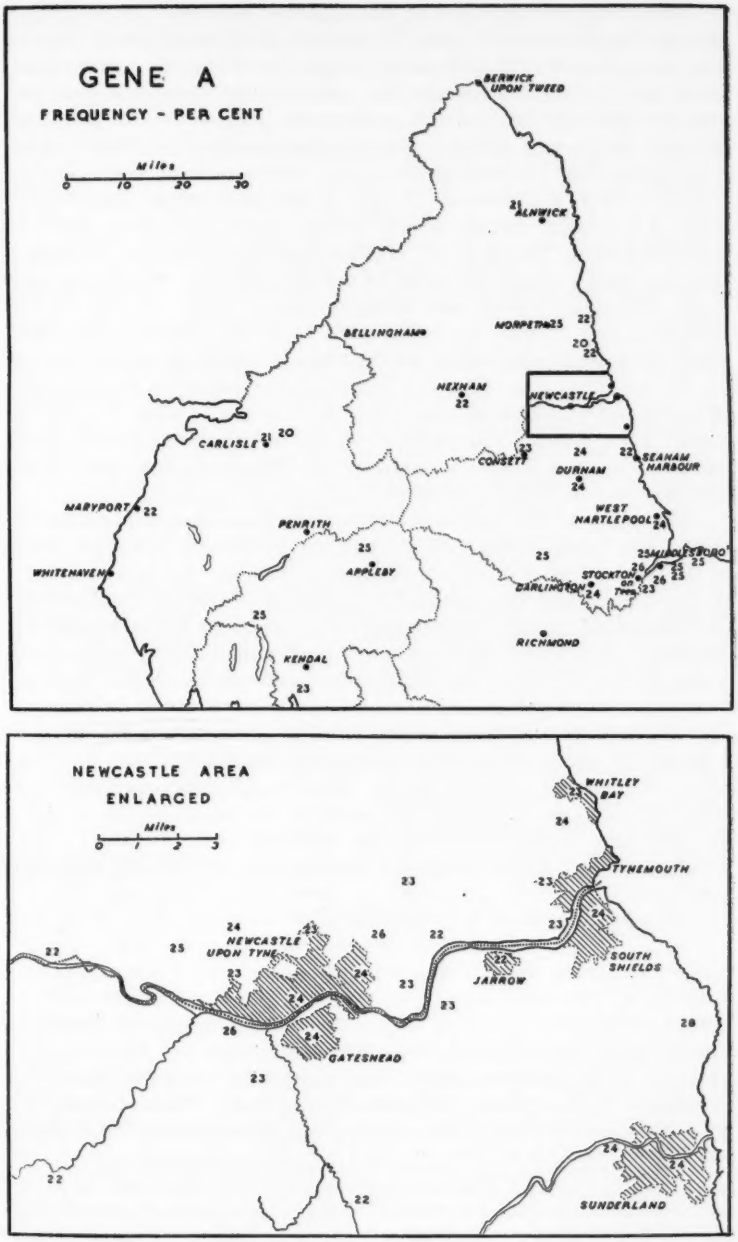
The line is shown on the O map, though in drawing it regard was also paid to the A percentages, and indeed principally to the A : O ratio. Starting in the west it is perfectly clear where the line must be drawn right up to the densely populated Newcastle area which is shown on an enlarged scale. Western Cumberland with its industrial areas lies to the north of the line. Is this due to relatively recent industrial immigration from Scotland and Ireland, or, as seems more probable, is it an indication of much older Celtic affinities? Carlisle lies definitely to the north; Penrith equally definitely to the south; Hexham lies north. The line turns northwards as it approaches Newcastle, but leaves Consett to the north. It then passes through the middle of the area of dense population. Newcastle itself represents an upward bulge, but the region of higher O and lower A comes in close on the western side of the City. Even more remarkably, the coastal area north of the River Tyne, including Whitley Bay, North Shields and Tynemouth, shows high O, as do Jarrow and Hebburn, south of the river, together with Wallsend and adjacent areas to the north.

To the north of Newcastle there is some irregularity. Morpeth (to which has been added Newbiggin) appears to belong to the region of lower O; the difference from the areas surrounding it being highly significant (χ^2, with Yates's correction, is 7·54). This district was one of the earliest to be dealt with, and the arrangement of the areas finally proved to be about the least satisfactory of any in the survey. It may be that Morpeth represents serologically a northward continuation of Newcastle, though it is difficult to think of any reason why this should be so. In fact, had the areas in the rather sparsely populated districts between them been differently demarcated and differently added, Morpeth might indeed have appeared as continuous with Newcastle. On the other hand one cannot help viewing the Morpeth figures with some suspicion and it may be that a fresh count on larger numbers would not confirm the frequencies now given. It is hoped to make such a count and the results will be reported in the next paper. For the time being, however, the town has been classified provisionally with the southern area of higher A.

South of the line, the area based on Ryhope and Seaham Harbour is high in O, showing 73 per cent.; but tested against the areas which surround it, the difference is not significant ($\chi^2 = 3{\cdot}04$). Consequently it does not seem necessary to show an island of high O here.

Naturally, it is possible to vary the exact position of the line somewhat without making either the northern or the southern area significantly heterogeneous. This hardly applies, however, outside the densely populated area and even here there is considerably less freedom of choice than might appear. In any event, however, it is drawn, the line must pass right through the densely populated area.


GENE O
FREQUENCY - PER CENT
Miles
0 10 20 30
BERWICK UPON TWEED
ALNWICK
BELLINGHAM
MORPETH
HEXHAM
NEWCASTLE
CARLISLE
CONSETT
DURHAM
SEAHAM HARBOUR
MARYPORT
PENRITH
WEST HARTLEPOOL
WHITEHAVEN
APPLEBY
STOCKTON
DARLINGTON
RICHMOND
KENDAL
NEWCASTLE AREA
ENLARGED
Miles
0 1 2 3
WHITLEY BAY
TYNEMOUTH
NEWCASTLE UPON TYNE
SOUTH SHIELDS
JARROW
GATESHEAD
SUNDERLAND


GENE A
FREQUENCY - PER CENT
Miles
0 10 20 30
BERWICK UPON TWEED
ALNWICK
BELLINGHAM
MORPETH
NEWCASTLE
HEXHAM
CARLISLE
CONSETT
DURHAM
SEAHAM HARBOUR
MARYPORT
PENRITH
WEST HARTLEPOOL
WHITEHAVEN
APPLEBY
STOCKTON
DARLINGTON
RICHMOND
KENDAL
NEWCASTLE AREA
ENLARGED
Miles
0 1 2 3
WHITLEY BAY
TYNEMOUTH
NEWCASTLE UPON TYNE
SOUTH SHIELDS
JARROW
GATESHEAD
SUNDERLAND

The strong evidence that in this region of England the northward rise in the frequency of gene O and the largely reciprocal fall in gene A is not a matter of gradual change, but is instead a step taking place at a remarkably definite line, prompts the suggestion that this may be generally true of the south-north gradient of England and Scotland taken as a whole ; namely, that the two countries can be divided into four (or perhaps five) areas within each of which there is relatively little variation in O and A, but that where they adjoin there is a marked change which occurs over a very short distance. It is interesting, therefore, to examine Fisher's figures for Yorkshire in some detail ; these are given in a communication which reported a sex difference (Fisher and Roberts, 1943). Only the figures for groups O and A have been published as it was thought that there might be inaccuracies which would make it unsafe to use the figures for the other groups. Hence it is necessary to work in terms of the ratio A/(O+A). (I was tempted to replace the O and A maps by a single map showing this useful ratio, but did not do so because gene frequency maps are now so universal.) Fisher's figures, with some calculations, are reproduced in table 4.

The area taken as a whole is highly heterogeneous, χ^2 for 10 degrees of freedom being 82·97. It is clear, however, that the great bulk of the variability is due to a difference between the southern part of the county and the remainder. Sheffield, Halifax, Huddersfield and Wakefield taken together give a χ^2 of 10·30 for 3 degrees of freedom. This is significant, but not highly so. The heterogeneity is due to the relatively low figure for Huddersfield, which must be included in the southern area because it lies to the south of Halifax. The whole of the rest of the area when these four centres are omitted is homogeneous, χ^2 being only 10·94 for 6 degrees of freedom. The large residual area, West Riding, less Skipton and Settle, ought perhaps to be omitted, as it may contain areas in the south of the county. Its omission, however, makes no appreciable difference. The A/(O+A) figure for the southern area is 48·0 and for the northern 45·8.

The line of division is remarkably clear although the centres are so large. It follows the River Humber and then the River Aire,* except that Leeds, which is situated on the Aire, and Bradford, slightly to the south of it, must be allotted to the northern part of the county. This is a line of outstanding importance in the history of England. Belloc (1912) points out that east of the Pennines the Aire was the natural barrier between north and south, the distance from the mountains to the marshes lining the estuary of the Humber being no more than twenty miles. The crossing was dominated by Pontefract.

* I have the impression that a number of years ago I read or heard it stated that the change from south to north in OA blood-group frequencies took place about the line of the River Wharfe (somewhat to the north of the Aire) ; but I have been unable to verify this or trace a reference.

On the other side of the Pennines there was a corresponding barrier formed by a natural belt of marshes along the valley of the Mersey, with a narrow gap at Stockport, dominated by Manchester. Whoever held Pontefract and Manchester held the gateways to the north, or south. It is not possible, however, to link up the line of the present survey with anything so definite, except that the River Tyne was also an important barrier.

TABLE 4

Fisher's data for groups O and A in Yorkshire, with an attempt at division into northern and southern portions

	O	A	A/(O+A) per cent.
Northern			
Leeds	11,359	9,805	46·33
Bradford	11,383	9,761	46·16
Hull	6,867	5,783	45·72
E. Riding	1,929	1,630	45·80
Vale of York	4,253	3,726	46·70
W. Riding (less Skipton and Settle)	23,406	19,488	45·43
Skipton and Settle	2,108	1,691	44·51
Total	61,305	51,884	45·84
Southern			
Sheffield	14,930	14,031	48·45
Wakefield	3,123	2,791	47·19
Huddersfield	3,245	2,806	46·37
Halifax	3,520	3,260	48·08
Total	24,818	22,888	47·98

Variation	D. of F.	χ^2	P
Within northern areas	6	*10·94*	0·090
Within southern areas	3	*10·30*	0·016
Between northern and southern areas	1	*61·73*	<0·0001
Within whole area	10	*82·97*	<0·0001

The AO figure for the 4 centres comprising southern Yorkshire is 48·0; this includes the large Sheffield area with a ratio of 48·4. These figures are very little lower than those for southern England. Fisher and Taylor's (1940) figure for south-eastern England is 48·8; Dobson and Ikin's (1946) for Cambridge is 48·7. Mine (Roberts, 1948) for the southwest is 49·7; but I suspect, for reasons given in that paper, that it is a little too high. Thus it seems clear that there is very little change in the proportion of genes O and A over an area which extends from the English Channel as far north as Sheffield, Halifax and Wakefield. Information is not yet sufficient to decide

whether a corresponding line can be drawn west of the Pennines and where it would lie. It would be interesting should it prove to pass through the Manchester area.

Fisher's northern Yorkshire area adjoins the southern part of the region described in the present paper. The AO ratio is 45·8, which turns out to be identical to three figures with an area comprising Darlington, Stockton-on-Tees, Middlesborough and West Hartlepool (Areas 58, 59 and 64 to 76 inclusive of table 2). The rest of the area south of the line on the O map gives 44·9, which is not significantly different from that for the area just defined ($\chi^2 = 2{\cdot}00$). Thus there is practically no change in O and A over a region which includes Leeds, Bradford and Hull and extends as far north as (most of) Newcastle.

Such figures as are available suggest that the AO ratio for most of Scotland is not very different from that found in the northern area of the present paper. Fisher and Taylor's (1940) data give 39·7. In the Highlands there is a further fall with certain areas showing very low values.

Mourant and Watkin (1952) in their maps of Western Europe show isogene lines at intervals of 5 per cent. The scale is, of course, small and it was intended to display broad trends only, but on the results of the present inquiry it would seem that the O isogene of 70 is drawn too far to the south. Incidentally, if the arguments of this section are accepted, it becomes difficult to draw isogene maps at all for Britain. It will be necessary rather to separate large areas and allot a figure to each instead of to the lines of division between them.

One last question remains: is there any evidence of local variability underlying the apparent homogeneity of the two areas into which the region is divided? Apparently there is not; O and A are quite unlike B in this respect, as is shown in the following section. One of the 52 areas does, however, stand out (45+47 of table 2); this is situated on the northern outskirts of Sunderland and includes Fulwell, Roker, Monswearmouth and Southwick, together with an area round Whitburn to the north. The frequency of O is 66 and of A 28 (the figures have been placed a little too far north on the map). Actually there were 410 O's and 422 A's, giving $A/(O+A) = 50{\cdot}7$, a ratio which differs with high significance from that of the surrounding areas taken together ($\chi^2 = 13{\cdot}68$). Fortunately the Sunderland area can be examined further, because Miss P. McCracken, the organising secretary in that area, has made an alternative division in the light of local knowledge. Her areas were coded and punched on the cards. The analysis will be made later and reported in another paper, dealing principally with family names, in which paper a more detailed examination of the whole region will be undertaken. With this one exception, the figures of the O and A maps do not suggest non-significant local variations which might be established with larger numbers. This is very different from the B map. Apparently,

therefore, in this region genes O and A do not display much local variability that cannot be accounted for by the transition from one area to another as part of the general south-north gradient of Britain.

(ii) *Frequency of gene B*

It has been usual to think of gene B as showing little variation in frequency throughout Britain. The figure of 6 per cent. very fairly represents England, or any reasonably large part of it. There is a slight but real rise to 7 in Scotland, Ireland and North Wales. Apart from this there is little or nothing in the way of a gradient. Recently, Watkin (Mourant and Watkin, 1952) has demonstrated some remarkable fluctuations of B in Wales, with very high frequencies in three areas described by Fleure as showing evidence of palæolithic survival. Moreover, in other areas in Wales and the Welsh Border there seems to be appreciable variability.

In the present study the 52 basic areas are highly heterogeneous, the B gene frequencies ranging from 4·9 to 8·5 per cent. It seems impossible, however, to discern any general gradient comparable to that shown by O and A. It is a question, rather, of apparently capricious local variations from place to place. It is not difficult, however, to single out the relatively few areas which are responsible for most of the heterogeneity. An analysis is shown in table 5.

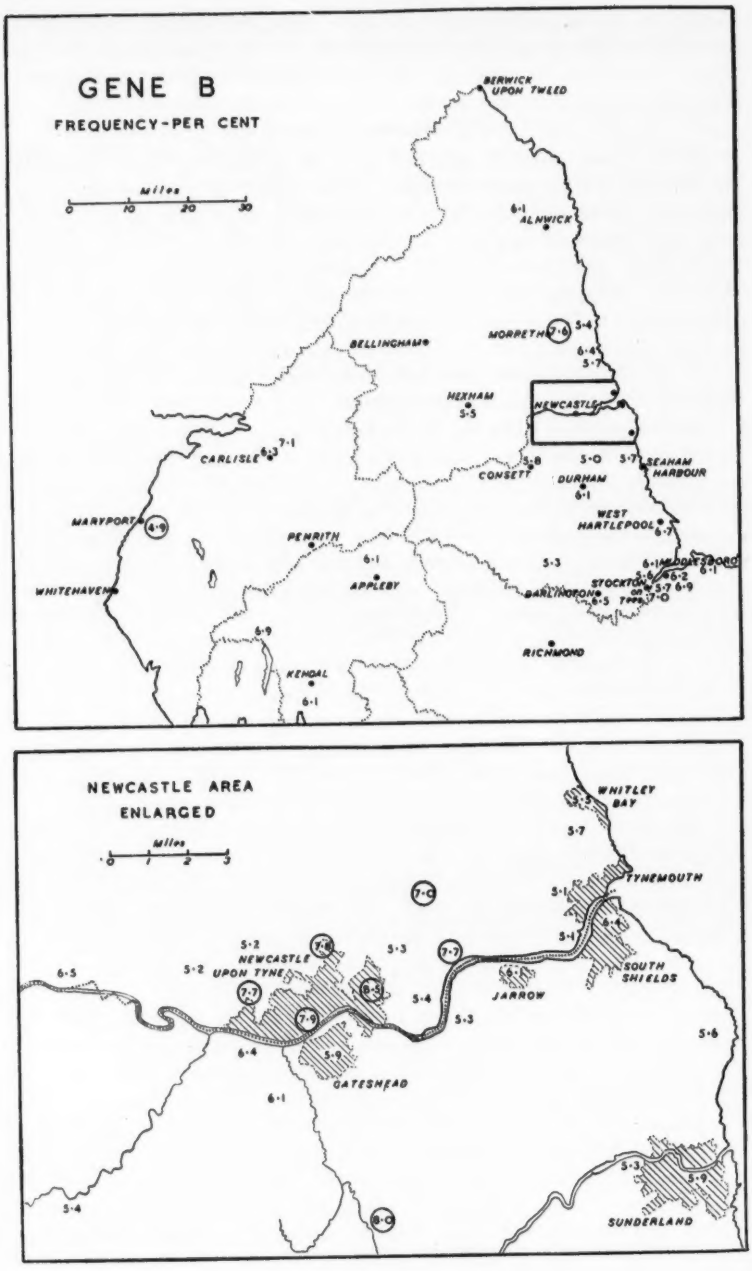
TABLE 5

Analysis of the 52 areas in respect of the variability of the frequency of B

	Area nos. (table 2)	No. of donors	No. of areas	(B+AB)/(O+A+B+AB) per cent.
1. Maryport . .	(83, 84, 85)	1,321	1	9·92
2. Chester-le-Street .	40	679	1	16·20
3. 7 contiguous N/c areas	(4, 5), (16, 17), 18, 22, (23, 24), 16, 29	8,421	7	14·06
4. Remainder . .	...	44,158	43	11·47
Total . . .	...	54,579	52	11·89

Variation	D. of F.	χ^2	P
1. Maryport	...	...	...
2. Chester-le-Street . . .	...	...	...
3. 7 contiguous N/c areas . .	6	*5·67*	0·46
4. Remainder . . .	42	*44·19*	0·39
Between the 4 groups . . .	3	*61·83*	<0·0001
Between all areas . . .	51	*111·69*	<0·0001

Note.—The figure 8·5 on the B map is that for Newcastle East. This is a residual area and so is continuous with the area shown as 7·7.


GENE B
FREQUENCY-PER CENT
Miles
0 10 20 30
BERWICK UPON TWEED
ALNWICK
6·1
MORPETH
7·0
5·4
6·4
5·7
BELLINGHAM
HEXHAM
5·5
NEWCASTLE
CARLISLE
6·3
7·1
5·8
5·0
5·7
SEAHAM HARBOUR
CONSETT
DURHAM
6·1
WEST HARTLEPOOL
6·7
MARYPORT
6·9
PENRITH
6·1
5·3
WHITEHAVEN
APPLEBY
STOCKTON ON TEES
5·7
6·9
6·2
DARLINGTON
6·5
7·0
RICHMOND
6·9
KENDAL
6·1
NEWCASTLE AREA ENLARGED
Miles
0 1 2 3
WHITLEY BAY
5·5
5·7
TYNEMOUTH
7·0
5·1
6·1
5·2
NEWCASTLE UPON TYNE
7·6
5·3
7·7
6·5
5·2
7·7
8·3
5·4
JARROW
SOUTH SHIELDS
7·0
5·3
5·6
6·4
5·9
GATESHEAD
6·1
5·4
8·0
5·3
5·9
SUNDERLAND

One area which stands out is the Cumberland coast, with the lowest B frequency of any area. These 1321 donors are made up by the addition of three areas at the 300 donor stage, as is shown in table 2, and it may be noted that the frequencies are closely similar in all three. If (B+AB)/(O+A+B+AB) for this area is compared with that for all the other donors west of the Pennines, the difference is significant ($\chi^2 = 5{\cdot}09$).

Centred on Newcastle is a group of six areas of very high B, as specified in table 5. To these must be added Morpeth, which, as was shown in the preceding section, may be provisionally considered as contiguous with Newcastle. The seven areas, though they are contiguous, do not combine into any kind of rounded whole which might be taken to represent the city, still less the greater Newcastle area. For example, the ratio for Gateshead, just across the river, differs significantly from that for the four divisions of Newcastle ($\chi^2 = 9{\cdot}64$). Similarly, the Denton area, embedded in Newcastle West, is significantly lower in B. As between themselves the seven areas of high B are homogeneous.

Chester-le-Street with Birtley shows the very high frequency of 8·0 per cent. Though quite near Newcastle, it is not contiguous with the seven areas just described, being separated from them by Gateshead and adjoining areas. Comparing the Chester-le-Street area with the areas surrounding it taken together, the difference is highly significant ($\chi^2 = 15{\cdot}83$). It is thus a circumscribed island of very high B.

Having deducted the foregoing 9 areas, the remaining 43 are not significantly heterogeneous, as is shown in table 5, and there is little suggestion of any other wide variations. Nevertheless, the B map does suggest that throughout the region there are other local variations, which might become significant with larger numbers. To take an example, a composite area comprising Middlesborough, Stockton-on-Tees, Darlington and West Hartlepool is significantly higher in B than the area adjoining it and comprising Bishop Auckland, Durham, Stanley and Seaham Harbour ($\chi^2 = 5{\cdot}11$).

Thus it would appear, if the northern region is representative of the country as a whole, that underlying the apparent constancy of B frequency there is a complex pattern of local variation. The apparent constancy is due to the absence of general gradients, so that if areas large enough to smooth out local variations are counted, much the same figure emerges from any part of the country.

Populations may presumably grow unlike, for even in industrial areas there is some measure of effective semi-isolation. As has been well said, large cities and towns, apart perhaps from their centres, are really collections of villages. Presumably too a gene like B of relatively low frequency is more subject to chance effects producing higher or lower frequencies than are genes like O and A, which are common everywhere.

(iii) *Conclusion on geographical variation, with a word on social differences and on urban and rural areas*

Any study of gradients and more particularly of heterogeneity in general is related to the numbers available for study. With relatively small numbers gradients can only be worked out very broadly and even wide local fluctuations over small areas may not be discerned at all. Each addition to numbers permits analysis at a new level ; and it is always possible that at some further stage a hitherto unsuspected pattern of variability may emerge. Who can say what might be revealed should numbers become so large that very small areas can be compared and half per cent. differences can be established ? Nevertheless, on the relatively large numbers of the present study fairly definite conclusions can be drawn.

Variations in the frequencies of genes O and A, which are largely reciprocal, depend upon the general change which is found as one passes from south to north, or west into North Wales. The change is apparently not a gradual one, but takes place in a series of steps. In the regions where the steps take place there are big variations between places situated quite close together, though when numbers are large enough it is likely that fairly definite lines demarcating the main areas can be drawn without much difficulty. The first line of division is situated about the line of the River Aire in Yorkshire. A corresponding line may be established in Lancashire, but the counts are at present insufficient to make sure of this. The next step is that illustrated in the O map. The third may be found to separate the Highlands from the Lowlands in Scotland ; and there are indications either of a further change within the Highlands, or perhaps of islands of extremely high O. The variability within the regions so separated is relatively small. Apart from the general gradient just described there appears to be little local variability in the frequencies of O and A. If such local variability does exist it must be confined to very small differences which could only be demonstrated by larger numbers than are available at present. This does not mean, however, that special areas, with a special history, may not show differences, for example Watkin's (1951) high A in an area of Pembrokeshire and about Chester, which may reflect Viking settlement.

The frequency of the B gene shows exactly the opposite kind of variability. There is very little in the way of any general gradient, apart from a one per cent. rise in Scotland, Ireland and North Wales. On the other hand there is relatively great local fluctuation from place to place, sometimes over quite small areas. Future work will no doubt show whether these variations can be related to history and geography or whether it is more a matter of chance differences.

It would seem from the data of the present paper that there is little if any difference between urban and rural areas in the same locality. Migration must have a diluting effect and no doubt the

variations found to-day are smaller than those that once existed; but it would seem that urbanisation is too recent a process to have established much relation to blood-group frequencies. The town-dwellers of to-day are but the descendants of the countrymen of four or five generations ago. Apart from the relatively high A of most of Newcastle, which may represent a northward extension into a more rural area, it is in fact remarkable how similar are the frequencies in rural and semi-rural areas to those of the towns they contain. It is hoped to give more evidence on this point in a further paper.

Nor is there any evidence that social differences are reflected by blood-group frequencies. There is some indirect information on this in the present data. Thus no two parts of a city could differ more in social conditions than do Newcastle East and Newcastle North; yet there is no sign of any corresponding difference in blood-groups. So presumably social differences also are too recent and too inconstant to have established any appreciable relation to the frequencies of the ABO genes. Further observations might be useful, particularly an occupational classification of donors, but one question at least which this pilot enquiry was designed to elucidate has been answered: it is well worth while pursuing the fluctuations of genes through the apparent complexities of the factories, the offices, the slag-heaps and the pitheads.

4. SPECIAL COMPARISONS

(i) *A special panel of donors at Newcastle*

Employees of the Ministry of National Insurance at Newcastle-upon-Tyne formed a special panel whose record cards were kept separately. In the counts of this paper they have been allotted to the various areas according to home address. An indication was, however, punched on the cards and so it was possible to discover whether their blood-group frequencies differed from those of the areas in which they are living. It was originally thought that they might include enough donors of non-local origin to affect the frequencies, though later enquiries by Mr Holland showed that this was not so. But in any event it is worth looking at a special occupational group to see whether it differs from the rest of the population.

The result is shown in table 6. Those whose homes are north of the line on the O map are closely similar in O and A to the rest of the population of that area; those whose homes lie south of the line resemble the donors of the southern part of the Region. In the same way those living within the 8 areas of high B, as defined in table 5, are high in B; those living outside the 8 areas share the relatively low B of the remaining homogeneous 43 areas of Table 5. Apparently, then, a special occupational grouping is not associated with any difference in frequencies as compared with other donors living in the same localities.

TABLE 6

Comparison of a special panel, employees of the Ministry of National Insurance, with the remainder, by areas

	A/(O+A)					
	Northern area (table 3)			*Southern area* (table 3)		
	No. (O+A)	A/(O+A)	χ^2	No. (O+A)	A/(O+A)	χ^2
M.N.I. . .	280	42·50	0·09	576	46·01	0·13
Remainder . .	16,985	41·41		30,249	45·17	

	(B+AB)/(O+A+B+AB)					
	High B areas (table 5)			*Low B areas* (table 5)		
	No.	(B+AB)/(O+A+B+AB)	χ^2	No.	(B+AB)/(O+A+B+AB)	χ^2
M.N.I. . .	459	13·07	0·43	514	11·09	0·03
Remainder . .	8,641	14·28		44,965	11·43	

(ii) *A comparison of the sexes*

Some years ago, when the material from Yorkshire summarised in table 4 was being examined by Sir Ronald Fisher, he noted that at an unexpectedly large number of the centres there were proportionately more women O's than men and more men A's than women. The difference of 0·51 per cent. in A/(O+A) was significant. In a count based on the six south-western counties of England I found a somewhat larger difference, namely 0·90 per cent., which was highly significant. On the other hand a large and undoubtedly reliable sample obtained by Dr Janet Vaughan at Slough showed no such difference, the ratio being slightly, though non-significantly, higher in women. It was thought possible, however, that the discrepancy might be due to the constant influx into the London area from Scotland, Ireland, Wales and the North of immigrants with a lower percentage of A and a preponderance of men. These findings have been reported (Fisher and Roberts, 1943).

As shown in table 7, the present material does not reveal any sex difference, the ratio A/(O+A) being closely similar in the area taken as a whole, and in the area of high O and the area of low O taken separately. All differences are well below the level of significance.

The sex of some 800 donors had not been recorded, half belonging to the Carlisle resigned panel, which, as is described in the next section, had to be reconstructed from the bleeding sheets. The 800 donors are omitted in the figures given in Table 7. Actually, all can be counted as men or all as women without affecting the conclusion.

TABLE 7

Comparison of the sexes in regard to the relative proportions of O and A

	A/(O+A)		
	Women	Men	χ^2
Northern area . . .	41·83	41·54	*0·132*
Southern area . . .	45·06	45·28	*0·138*
Total . . .	43·90	43·92	*0·001*

Note: The two areas here are nearly but not quite the same as Table 3.

5. SUMMARY

1. A pilot survey has been carried out to throw light on :—
   (*a*) The use that can be made for anthropological purposes of the records of the National Blood Transfusion Service.
   (*b*) Any difficulties that might make their use unsafe in certain circumstances.
   (*c*) Additional points of value that might be incorporated in future records.
   (*d*) Methods of making the analysis economically.
   (*e*) The extent to which local fluctuations in frequencies occur and hence the numbers and the detail that it is profitable to include, especially in densely populated areas.

2. The area selected was the Northern Region comprising the counties of Northumberland, Durham, Cumberland and Westmorland, with part of the North Riding of Yorkshire. So in addition to the points mentioned above, it was hoped that the survey would be of value as a detailed mapping of an interesting area.

3. In examining heterogeneity throughout the area two simple and basic $2 \times N$ comparisons were used : the ratio A/(O+A), which covers the largely reciprocal fluctuations of genes O and A ; the ratio (B+AB)/(O+A+B+AB), which is closely related to the frequency of gene B. For the first comparison areas must have a minimum number of donors of 20 ; for the second 70.

4. The geographical sub-division was made as fine as possible. Single towns, parts of towns and villages were kept separate when

possible. The total count yielded 54,579 donors, divided into 321 areas, each containing at least 20 donors.

5. Both ratios showed highly significant heterogeneity. It was found that the original 321 areas could be added together, purely geographically, to form larger aggregates up to a stage when there were 52 areas, each containing at least 600 donors; at this stage, for both ratios, all the heterogeneity lay between the 52 areas and none within them. Further addition did not work, as at the next stage there was significant heterogeneity within areas. Accordingly the 52 homogeneous areas were used for subsequent comparisons, though the group frequencies are given for the previous stage of 90 areas.

6. Fisher's test for the reasonableness of group proportions for the 52 areas and for the total proved very satisfactory.

7. Nearly all the heterogeneity in the frequencies of genes O and A is accounted for by the south-north rise in O and fall in A. It is possible to draw a single line from east to west such that all the heterogeneity lies between the two areas so separated and none within them. The line passes through the middle of the densely populated Newcastle area.

8. The change is thus not a gradual one, but takes place at a definite line. A comparison with Fisher's figures for groups O and A in Yorkshire shows almost identical figures for the northern part of Yorkshire and the adjoining southern part of the region surveyed in this paper. In Yorkshire also the change in frequencies takes place at a very definite line, south of which the figures are very similar to those of southern England generally. It is likely that the northern half of the North of England Region is little different from Scotland, until the Highlands are reached.

9. The fluctuations in the frequency of gene B show exactly the opposite kind of variation. There is no general gradient, but very marked fluctuation from place to place, sometimes over relatively small distances.

10. There is no indication of differences between rural and semi-rural areas and the towns they contain; nor is there any indication of social associations.

11. There is no sex difference in frequencies.

12. Points that may help in the planning and carrying out of future surveys are discussed in the Appendix. Donors may not resign in proportion to their blood-groups and so it is very important that the records of all resigned donors should be complete and available. Unless this is certain, only new registrations can be counted. All the indications point to the present record being a satisfactory body of material. It is certainly worth while including in counts for the British Isles as large numbers as possible and making as fine a sub-division as possible, even within large towns.

6. APPENDIX

Some lessons on the making of counts from the records of the National Blood Transfusion Service

1. In the practical business of establishing and maintaining a donor panel as the basis of a transfusion service, the point is soon reached when it becomes necessary to remove the cards of donors who are no longer active from the live panel which is in continual use. When donors die, or move away from the region, or resign, or fail to respond to repeated requests to attend for bleeding, their cards are removed and added to the resigned panel. It might be anticipated that the proportion of resignations is not necessarily the same in the different blood-groups. In earlier years particularly, donors of the different groups were called up with unequal frequency ; and they may tend to resign if they are not called up often enough, or, alternatively, too often. A plan is needed for dealing with donors who fail to respond. In the North of England the practice has been that if a donor ignores three successive requests without writing or telephoning an explanation he or she is removed from the active panel and the card is added to the resigned panel. Thus if the frequency of calling up is different for different groups, defaulting donors of some groups will be removed in a shorter time than will those of others.

A comparison was made when the cards of the Transfusion Service panels for the four divisions of Newcastle had been copied, together with those of Gateshead. By this time some 5000 other donors had been included, drawn from centres in various parts of the region. This classification cuts across that of table 2, but will serve for the present purpose. The results are shown in table 8. The live and

TABLE 8

Comparison of live and resigned panels, showing that each alone may give impossible frequencies, but that when added together the frequencies are unexceptionable

	No.	Group frequencies per cent.				Gene frequencies per cent.			AB Obs.-Exp.	χ^2
		O	A	B	AB	O	A	B		
		Four divisions of Newcastle and Gateshead								
Live	5,727	42·68	40·39	14·07	2·86	...	...	...	−131·83	*39·43*
Resigned	7,845	52·95	37·58	6·56	2·11	...	...	...	+74·23	*26·55*
Total	13,572	48·61	38·76	9·73	2·89	69·6	23·7	6·7	−37·49	*2·33*
		Other areas counted up to that stage								
Live	3,169	51·85	36·45	9·59	2·11	...	...	...	−21·78	*3·91*
Resigned	1,786	54·09	34·88	8·23	2·80	...	...	...	+9·93	*1·84*
Total	4,955	52·65	35·88	9·10	2·36	72·5	21·5	6·0	−11·49	*0·76*

resigned panels for Newcastle and Gateshead taken separately give entirely impossible group frequencies, whereas when they are added together the gene frequencies and the χ^2's are perfectly reasonable. Although in the light of the points mentioned above, considerable discrepancies were not unexpected, their magnitude surprised the officers of the region, who do not feel that any suggested explanation is fully adequate.

On the other hand the 5000 live and resigned donors from other areas do not differ greatly, though even amongst them the live panel gives a significant χ^2. The difference from the Newcastle area may be due to the fact that donors on the smaller panels making up the 5000 were called for bleeding without the same degree of selection. With a small panel it was necessary to call all the donors, of whatever group, on each occasion in order to ensure an economic session. But even if there may not always be a serious difference, or indeed any difference, between live and resigned panels, this cannot be guaranteed without making a count of both.

The completeness of the resigned panel is thus the most important factor in determining whether or not the records of the past can be used with safety. If there is any possibility that some of the cards of resigned donors may have been destroyed, the whole record is unusable for anthropological purposes. And it is not only a question of destruction. The cards of resigned donors may be stored in a way that makes it possible for some of them to be overlooked when a count is attempted. Only if it is reasonably certain that the whole record, live and resigned, is complete, can any existing body of data be used for analysis. Failing this the only plan is to fix a date and count new registrations from that time onwards.

The whole body of data from the Northern Region shows, as it was hoped would be the case, every indication of completeness, but at one centre, Carlisle, it was found that the resigned cards had been destroyed. Sooner than omit this important area and so spoil the completeness of the analysis for the Region as a whole, it was decided to reconstruct the resigned panel from the original bleeding sheets, that is, the detailed records of all donations of blood, these still being available. It naturally proved to be a most troublesome and time-consuming task, not to be recommended for any purpose other than the filling in of a gap, as in this instance. It was necessary to prepare a card index from all the bleeding sheets, as well as of the live panel, going back to the beginnings of the Service, and then to attempt to eliminate duplicates. The magnitude of the work will be realised when it is mentioned that the original index (with many duplicates eliminated as the work proceeded) amounted to 8000 cards. The difficulties due to similar common names, marriage, change of address and so forth will be obvious enough. During the last stages Mr C. H. Holland, the Regional Donor Officer, gave his help and I trust that the final index of resigned donors contains relatively few donors counted twice or instances of two separate donors considered to be the same person. There is a check on the second possibility. No attention was paid to blood-group in making the decision and when the original 8000 had been reduced to a quarter of that number it was found that in only 8 instances were the groups different in donors supposed to be the same. The first source of error, that is, counting a single donor twice, cannot be directly checked, but I hope that it too has led to little inaccuracy. The Carlisle figures taken as a whole, and broken down in different ways, revealed no suspicious anomaly, so for the sake of completeness they are included, in the hope, which I trust is not unreasonable, that no appreciable error has been introduced.

2. The borrowing of the original cards for copying is to be avoided. The present pilot survey was only made possible by the willingness of the Region to lend them ; but it put the officers to a great deal of trouble. At the receiving end, batches of live cards had to be dealt with very quickly at times and there was always anxiety lest some of the cards, which were irreplaceable, might go astray. A count based on the accumulated material of the past is very difficult to carry out except by someone working locally and preferably someone in the Transfusion Service.

The ideal plan, which it is hoped will now be operated for newly registered donors, involves the preparation of copies by the Regions at the time of registration for transmission to and retention by the Nuffield Blood Group Centre at the Royal Anthropological Institute.

3. The breaking-down of the material by geographical area was the most difficult and time-consuming part of the present enquiry. To do so *ad hoc* for every fresh survey is clearly not to be recommended. The only indication of area on the cards is postal address so this must be accepted as the basis of classification. What is required is a code made once and for all, so that any address can be immediately turned into a code number. The code must be hierarchical, so that a count can be made at any desired level of sub-division. The best plan would then be to code fully in the first place down to the finest sub-division provided and to prepare the tables (or better, summary cards) accordingly. Coarser grouping could then be made by addition. The code should be based on numbers of people rather than on square miles, which is presumably what postal address essentially does. The postal districts of large cities should be adequate for tracing possible variations within them. Coding in this way should reduce the work to a minimum and make it possible to add new counts to previous ones.

4. In the present survey the use of punched card methods was a great convenience. The cards had to be copied anyway and punching and verification, using an electric automatic key punch, was quicker than hand copying would have been. The ultimate sorting and counting was carried out very conveniently on Powers-Samas sorter-counters and it might be mentioned that there was never any trouble about exact agreement of totals and sub-totals. Nor was any card wrecked so badly that the information could not be reconstituted and a new card punched. The punching and verification was carried out by my assistants, Miss Hill and Miss Ralph, and the Powers-Samas company kindly allowed us to take batches of cards to their Office to put through a comparator, so avoiding the necessity for sight reading of the verification. The sorting and counting was also done by ourselves, and it should be added that the scheme was workable only because we had the machines, both automatic punch and sorter-counters, available at the School of Hygiene.

Nevertheless, I am doubtful whether punched card methods are needed when copies of the original cards are provided and can be retained. Once the area code number has been entered, the copies can be hand sorted and counted as desired and tables or summary cards prepared once and for all, without it being necessary to repeat the copying process. This would cover the ordinary routine count, which involves so few and such simple particulars ; though for special pieces of work, for example, an analysis using family names, punched card methods might be considered.

5. If one considers that much of the present variation in blood-group frequencies is probably a diluted version of the variation that once existed, then anything that can push the count backwards in time, as it were, should be of value. One way of doing this is to record place of birth as well as present residence. Place of birth of father and of mother is an additional refinement. It is not suggested, however, that these particulars should replace present residence as the basis of the count ; rather, they should be used for alternative counts, because the place of birth of the donor and still more of father and mother will often not be specified with the accuracy needed for fine geographical sub-division.

Classification by family name, as a partial measure of racial origin, has proved useful in many surveys and will probably do so in this one. Here a refinement is to record the maiden name of married women and the maiden name of mother. Once again, however, I think that I should state my personal opinion that basic counts should refer to all donors in each area, except for readily identifiable large bodies of clearly temporary and non-local residents, such as military personnel at a camp. The refinements add greatly to the value of surveys and the results may be largely discussed in terms of them ; but it is the total basic counts that will be most easily comparable over periods of time and sometimes as between area and area when different workers are concerned.

During the course of the work there have been discussions with the authorities of the National Blood Transfusion Service on the possibility of adding items of

information of anthropological value. As a result certain additional particulars have already been incorporated in the record cards.

6. It is evident that for the present at least it will be profitable to analyse material from all over the country, using as large numbers as are available and with as fine a geographical sub-division as is practicable. It is impossible to say without trying it what other large towns may not show the heterogeneity of the Newcastle area ; only when it is established that no such variation exists should the count be stopped or the sub-division made coarser. If the evidence is reviewed from time to time it will become clear when in any part of the country addition to existing counts is unlikely to yield much further information ; but that time is not yet. There is still much work to do in filling in the details of the ABO maps of the British Isles, to say nothing of the other blood-group systems. This will not only give the general picture, but will help to indicate particular areas where more intensive work would be profitable. Special investigations can then be planned, using numbers much larger than those even of the Transfusion Service and incorporating additional information that cannot be obtained from donors' record cards.

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HETEROCHROMATIC AND EUCHROMATIC GENES ACTING ON QUANTITATIVE CHARACTERS, IN *D. MELANOGASTER*

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1. INTRODUCTION

THE last paper by the senior author (1951) on the action exerted by the Y chromosome pointed out that, comparing stocks differing merely by the Y chromosome (known to be heterochromatic), size differences can be observed in the wing cells and in the eye corneæ. The Y chromosome's effect was then proved by comparing the differences between sexes ; no comparison was made between males of different homogeneous stocks, *i.e.* by testing whether the different Y's produce an heterogeneity between males of different stocks. Nevertheless the conclusion seemed obvious, that the Y chromosome of *D. melanogaster*, except in a few loci, acts quantitatively on small characters ; this agrees fundamentally with the point of view expressed by Mather (1944, 1949). Further, the difference between the wild Y's was interpreted as being produced by different systems of genes interacting together.

The aim of the present work is now :—

(i) To complete the demonstration of the Y chromosome's action, using a new method which will be given below.

(ii) To secure further evidence in favour of the polygenic nature of the factors located in the Y.

(iii) To compare the action of the Y chromosome with that of major genes located in euchromatin, in order to see whether discrimination is possible between heterochromatic and euchromatic genes.

2. MATERIAL AND METHODS

The material used in this investigation * is composed of the same group of stocks investigated previously (1951), namely : Crkwenika—Luino—Oerlikon—Oregon.

All the stocks had been made homogeneous for all chromosomes, except the Y and the IVth pair.

It seemed important to us to compare the measurements of the characters made at two different times, *i.e.* some years ago and after an interval of nearly 20-30 generations ; and to repeat afterwards the homogeneisation. This procedure was likely to indicate whether different mechanisms (mutation, recombination, selection, drift) can, as a whole, change a polygenic genotype.

* We are indebted to Miss E. Bassi for her valuable help in making preparations and counting, and to Mr H. Rees for revision in the English.

With this aim, both characters already investigated (frequency of wing hairs, area of the corneæ) were measured, (*a*) before the homogenisation, (*b*) immediately after the homogeneisation.

The homogenisation itself was carried out using the balanced stock : $\frac{Cl/B}{+}$ $\frac{Pm}{Cy\ L}$ $\frac{H}{Sb\ Me}$, and Oregon as background, obtaining the following genotypes :

MALES			FEMALES		
$\frac{X^{Oregon}}{Y^{Oregon}}$	II Chr^{Oregon} / II Chr^{Oregon}	III Chr^{Oregon} / III Chr^{Oregon}			
$\frac{X^{Oregon}}{Y^{Crkwenika}}$	,,	,,	always :		
$\frac{X^{Oregon}}{Y^{Oerlikon}}$	,,	,,	$\frac{X^{Oregon}}{X^{Oregon}}$	$\frac{II^{Oregon}}{II^{Oregon}}$	$\frac{III^{Oregon}}{III^{Oregon}}$
$\frac{X^{Oregon}}{Y^{Luino}}$	,,	,,			

In the present investigation in order to obtain a comparable scaling for both wing cells and corneal areas, the reciprocal of the number of hairs counted in a given surface near cross vein II of the wing was used. Taking the area equal to 1, the quantity $\frac{l}{n}$, where n is the number of hairs, corresponds to the mean area, containing one hair, *i.e.* it is proportional to the mean area of the cells included in the standard surface.

The investigated characters are thus : the mean surface area of the wing cells and the mean surface area of the corneæ of the compound eye ; each cornea comprises a fixed number (2 od 4) cells (see Barigozzi, 1951).

The comparisons between samples were always made by means of F (variance ratio) to test the homogeneity or the heterogeneity of a group of flies supposed to have identical or different genotypes.

3. OBSERVATIONS

(a) *Variations of intensity in wing hairs during four years* (1948-1952) have been scored (see table 1). The differences between sexes are

TABLE 1

Differences between sexes and their errors

	1948	1949	1951	1952
Oregon . . .	4·66±0·611	2·70±0·239	2·04±0·460	1·87±0·306
Oerlikon . .	3·30±0·209	1·44±0·227	2·36±0·422	1·80±0·481
Crkwenika . .	1·42±0·251	...	2·25±0·4521	1·89±0·515
Luino . . .	*0·58±0·377*	*0·51±0·330*	1·59±0·464	*1·46±0·589*

N.B.—The differences in italics are not significant.

given, and also the standard errors, so as to make the data comparable for samples that are not all of exactly the same magnitude.

It is worth noting that between 1948 and 1949, and again between 1951 and 1952 a substitution of genomes (oregonisation) took place,

marked in the table with double vertical lines. One recognises that a change occurs in each interval, which covers about 10 generations per year.

The change is not very big, but is sufficient to show :—

(i) that many genes (polygenes) must act on the character, that are capable of causing a continuous genotypical change ; the actual data appear discontinuous only because the observations also are discontinuous.

(ii) that the oregonisation, as a substitution of the entire genome, exerts an effect similar to the other mechanisms usually at work (mutation, recombination, etc.)

It is especially worth remarking on the data concerning Luino. The difference between sexes was not significant before and after the first oregonisation (1948-49), then became significant before the second oregonisation (1951), to become again insignificant afterwards.

From these facts it can be concluded that the character is influenced by different parts of the genome, and not only by the Y chromosome. A typical feature of the oregonisation (although not shown in the table) is that the change is suddenly produced. It is also worth noting that the oregonisation brings about an increase in the cell size in both sexes.

Furthermore it is important that the corresponding data on corneæ show similar trends to those on wing hairs.

(b) *Homogeneity and heterogeneity of the oregonised stocks.*—The working hypothesis used in this part of the investigation is the following : after a genome substitution, involving all chromosomes except the Y and the small IVth pair, the females must be practically alike in the different stocks, except for the IVth chromosomes, which might introduce a slight variation. The males, on the contrary, carrying a Y which is typical for each stock, may show differences between stocks.

To test this hypothesis, a comparison is necessary among males and females separately. It is also necessary to find out whether each stock is sufficiently homogeneous. From each stock, 10 cultures were made (kept at a temperature of 25°) and from each culture 20 individuals (10 males and 10 females) were investigated for the wing hairs ; and 4 individuals (2 males and 2 females) for the corneæ. The measurements were subjected to an analysis of variance, to test whether a sufficient degree of homogeneity within the stocks was reached.

Testing the variance between cultures against the variance within cultures by means of the F variance ratio (with 9 and 90 degrees of freedom for the wing cells and with 9 and 10 d.f. for the corneæ), gave low values for F. This shows that variations within each stock can be considered with high probability as not significant (see table 2). Note that the F value of the corneæ of the Luino females (3·5476)

reaches the level of significance at 5 per cent. (3·02), and that of Luino males for the wing cells (3·1866) is also significant.

This conclusion that the stocks are in general homogeneous, permits us to take the mean of each culture instead of the individual measurements, in order to compare the variance between stocks with the variance within stocks, which is the second step of our investigation. With regard to the significant difference between the Luino males for wing cell size, the fact that the oregonisation procedure was the same for all stocks indicates that this heterogeneity must depend on a heterogeneity of the response to environment of

TABLE 2

Variance ratios within Oregonised stocks $\frac{\text{Variance between cultures}}{\text{Variance within cultures}}$

	Wings (N = 100) D.F. : 9 ; 90		Corneæ (N = 20) D.F. : 9 ; 10	
	♂	♀	♂	♀
Oregon . . .	0·5222	0·6695	1·5857	0·4639
Crkwenika . .	1·6112	0·7413	1·001	2·6329
Oerlikon . .	0·9871	1·3447	0·5506	1·6516
Luino . . .	3·1866	0·7484	1·2756	3·5476

P always >5 per cent., except : males Luino for wing cells, where P<1 per cent.

the Luino males. This fact does not allow us to attribute to this mean the same significance as to the others, but does not affect the discrimination between stocks.

When the variance between stocks is tested against the variance within cultures of each stock, separately for females and males, the following results are obtained (table 3). The variation of areas for the wing cells does not show any significant heterogeneity for the females, while a highly significant heterogeneity is observed for the males. This means that, in agreement with expectation, the female genotype is fundamentally the same in all stocks, while this is not the case for the males. Since between stocks the main source of difference is the Y chromosome, it seems reasonable to interpret the discrepancies among the males for different stocks, as being due mainly to Y chromosome effects.

It is also worth noting that this effect is similar for both the characters studied. In addition, the fact that the wing cells show a high variability within the Luino males is a further evidence for different activities of the different Y's. The Y of Luino thus seems to be characterised by a weaker genotypic control of the character under consideration. The demonstration of the influence of the Y chromosome on cell size is in accordance with the previous observation made by one of us (Barigozzi, 1951).

If it seems justified to ascribe to the Y chromosome a prevalent

action in determining the high variation in cell size of the males, it does not follow that this variation depends solely on that. We cannot disregard the fact that in the procedure of homogeneisation

TABLE 3

	Wing cells—Females				Corneæ—Females			
	Crkwenika	Oerlikon	Oregon	Luino	Crkwenika	Oerlikon	Oregon	Luino
1	0·08920	0·0884	0·0909	0·0925	318·291	344·121	301·139	306·934
2	0·09009	0·0909	0·0943	0·0934	319·303	346·036	336·611	307·533
3	0·09009	0·0917	0·0943	0·0934	324·987	355·932	340·068	313·738
4	0·09170	0·0934	0·0943	0·0943	328·163	363·776	353·310	316·732
5	0·09250	0·0943	0·0952	0·0965	339·773	378·022	353·369	317·635
6	0·09250	0·0952	0·0952	0·0970	346·909	384·117	354·254	328·904
7	0·09340	0·0952	0·0961	0·0978	350·070	386·017	359·368	338·162
8	0·09340	0·0952	0·0961	0·0980	351·141	387·732	371·306	339·340
9	0·09520	0·0961	0·0970	0·0980	365·024	415·675	371·826	354·223
10	0·09800	0·1000	0·0980	0·1010	372·240	421·083	372·298	360·124
Means	0·092608	0·09404	0·09514	0·09619	341·590	378·251	351·354	328·332

D.F. : 3 ; 36. F = 1·738. P>5 per cent.

D.F. : 3 ; 36. F = 2·328. P>5 per cent.

	Males				Males			
1	0·0746	0·0763	0·0781	0·0793	262·146	307·286	306·001	262·961
2	0·0769	0·0775	0·0793	0·0800	289·565	308·386	308·692	271·080
3	0·0775	0·0781	0·0800	0·0800	296·733	325·380	309·663	293·092
4	0·0775	0·0800	0·0800	0·0819	297·224	326·214	310·655	293·268
5	0·0787	0·0806	0·0800	0·0854	302·182	328·727	317·016	298·324
6	0·0787	0·0813	0·0813	0·0854	302·605	331·242	323·286	304·548
7	0·0787	0·0819	0·0813	0·0854	304·899	331·487	326·813	304·971
8	0·0793	0·0819	0·0819	0·0869	319·047	332·183	350·325	311·568
9	0·0813	0·0826	0·0819	0·0884	330·132	347·508	356·207	321·797
10	0·0854	0·0833	0·0826	0·0909	332·282	348·097	359·525	338·938
Means	0·07886	0·08035	0·08064	0·08436	303·681	328·651	327·318	300·048

D.F. : 3 ; 36. F = 5·147. P<1 per cent.

D.F. : 3 ; 36. F = 5·979. P<1 per cent.

the chromosome IV was not controlled, and that some rare crossovers can also occur, when crossover preventing inversions are present. For that reason, we must be cautious in considering the variation shown by the females as being pure non-heritable. Meanwhile it cannot be denied that males and females show a degree of homogeneity which is substantially different.

(c) m^+ *and* m^2, *as oligogenes acting on cell size.*—The existence of the major gene *miniature* (m^2, Dobzhansky, 1929) acting on the cell size, permits us to analyse the relationships between this and the systems of polygenes previously considered.

The stock carrying m^2 was actually marked by the genotype : m^2 *g*. Crosses were made with the usual stocks in order to obtain

males carrying the different Y's and m^2 (I ; 36·1). These males are heterozygotes, having one-half of the chromosomes from the m^2 *g* stock and one-half from Oregon, except the Y chromosome, which is, respectively : Oregon, Luino, Oerlikon or Crkwenika.

The measurements made on the original m^2 *g* stock and on homozygous Oregon are given for comparison in table 4. It is clear that

TABLE 4

Comparison between males with m^+ *(Oregon) and with* m^2 *(40 individuals for the wing cells, and 10 for the corneæ)*

	Wing cells	Corneæ
Oregon (m^+ Y)	0·080640±0·0006108	327·318±6·582
Miniature^2 Y	0·037566±0·0008432	268·261±5·912
Difference	0·043074	59·057

the conclusions by Dobzhansky (1929) concerning the wing cells are confirmed, and further the results show that the gene also affects the eye corneæ.

The data for the heterozygous Y/m^2 *g* males are given in table 5 and show that a significant difference exists between stocks for the

TABLE 5

Males with m^2. *Each figure corresponds to the mean taken from 10 individuals for the wing cells, and from 3 individuals for the corneæ*

	Wing cells (No. of individuals : 50)			
	Crkwenika	Oerlikon	Oregon	Luino
1	0·03672	0·03603	0·03810	0·03889
2	0·03754	0·03634	0·03839	0·03914
3	0·03806	0·03686	0·03855	0·03964
4	0·03896	0·03733	0·03890	0·03982
5	0·03945	0·03792	0·04013	0·03992
Means .	0·038146	0·036896	0·038814	0·039482

D.F. : 3 ; 16. F = 9·444. P<1 per cent.

	Corneæ (No. of individuals : 15)			
1	279·738	282·436	277·645	274·883
2	282·553	291·768	278·470	283·470
3	306·763	301·501	297·555	284·557
4	306·979	304·734	303·681	285·577
5	329·559	315·730	316·574	294·171
Means .	301·118	299·233	294·785	284·531

D.F. = 3 ; 16. F = 1·211. P>5 per cent.

wing cells, but not for the corneæ. The conclusion is thus justified, that, when m^2 is present instead of the allele m^+, the polygenes of the Y chromosome modify the expression of the main gene as far as the wing cells are concerned, but not its effect on the corneæ.

Obviously, the data were obtained after a preliminary investigation to test the homogeneity within each stock. Beside the males, a corresponding number of females was also examined, to test whether the presence of m^2 in heterozygous condition is able to influence the phenotype. We conclude from this investigation that it is not strong.

It may be quoted, incidentally, that a semidominance of m^2 is found, since the values for mean wing cells and corneæ for Oregon females are respectively : 0·6695 ; μ^2 351·354 and for *miniature*^{2} females : wing cells, 0·4115 ; corneæ μ^2 291·687.

4. DISCUSSION

The data, which we are now going to discuss can be summarised as follows :—

(i) The stocks change over a series of generations (even when kept in the most constant culture conditions and with severe inbreeding) with respect of wing and cornea cell size in both sexes, and thus probably independently from the Y chromosome effect.

(ii) The Y chromosome, in agreement also with the previous investigation of the senior author, exerts an influence on the cell size of the male.

(iii) The pair of alleles m^+-m^2 (I ; 36·1) also acts on the cell size of both wings and corneæ, but more strongly than the Y chromosome and the other polygenes. The allele m^2 interferes in a peculiar way with the action of the Y chromosome, being effective on wing cells and not on corneæ.

Certain problems can now be separately discussed.

(a) *Activity of the Y chromosome as a system of polygenes.*—In a previous paper by the senior author (1951) some evidence was given supporting the view that in the Y chromosome a system of polygenes is at work. Actually, on the basis of one single factor, it would be difficult to explain the quantitative differences among stocks. The present work shows better evidence of the polygenic content of this chromosome ; namely, the changes observed in the Y's after a number of generations (table 1). It is clear that such changes can be better understood on a polygenic basis, owing to the mechanisms of genotypic variation, which can be accounted for primarily by mutation and crossing-over. The first phenomenon is rare for one single locus, but, after a relatively

short number of generations, may occur rather frequently if the number of genes involved is high. The variation by means of crossing over is also obviously understandable for the pairing segment between X and Y, assuming the presence of many genes located in the same chromosome, and acting in a similar manner.

These polygenes are considered to form a system in the sense that they work together on the cell size. The activity of such a system is not independent but modifies the effect of other factors. Among these, some can be considered as main genes according to Mather (1949), and up to now two of these have been determined. In the previous paper, a modification was described which is determined by the Y chromosome on the frequency of irregular eye facets, produced by the SB Me' inversion (chromosome III ; Di Pasquale, 1951). In the present paper, a similar modification was shown for the pair of alleles m^2-m^+, and thus acts in a very peculiar way. The allele m^+ (greater cells) is modified in its expression by the Y chromosome, while m^2 (smaller cells) can be modified only in its effect on wing cell size. It must be noted that it cannot be excluded that other factors work with m^2, since the stock carrying m^2 was not made homogeneous with the other stocks.

The genotypic variation of cell size must also depend on another element, *i.e.* the sex determining factors. Little is known about the localisation of these factors, but it is nonetheless certain that they act on cell size (and probably also on cell number), determining a difference in size between male and female. In the present work both cell measurements show a greater size in the females, but the data collected in table 1 show clearly that the difference in cell size between sexes can be modified to such extent by the Y chromosome, as to become negligible (*e.g.* the Luino stock at the 1st oregonisation).

The Y chromosome genes of *Drosophila melanogaster*, except for the few major genes located in it, must therefore be considered to act as a system of modifiers. The modification seems to control several characters beside the cell size, for example " mottling effect ", " Podoptera effect " (Goldschmidt *et al.*, 1951). This action leads to an understanding of the importance of the Y chromosome, for its possibility of adjusting the expression of oligogenic characters can be useful in selection, when several types of Y chromosomes are present in a population.

We do not dare, on the other hand, to extend the conclusions reached for *D. melanogaster* to other species, until appropriate data are collected.

One last point to be considered, concerns the action of polygenes located in the Y chromosome.

The question shows two main sides : that of the pleiotropism, and that of the actual mode of action on the cell size.

How to understand pleiotropism in the character studied has been discussed previously (Barigozzi, 1951) and we still incline to

consider the present case (*i.e.* action on cell size) as a spurious example. The reason is that an effect on all cells affect automatically all organs by means of one single mechanism, and not by means of different modes of action, as it occurs in the true pleiotropism.

The action of the different genes on cell size can be understood in different ways.

The genes located in the Y chromosome can support the view put forward by Caspersson (1950), and already discussed previously (Barigozzi, 1951), *i.e.* of a participation in the protein synthesis during the growth processes. The action played by the pair m^{+}-m^{2} cannot be put in the same category.

(b) *Polygenes and major genes.*—An important question raised several times concerns the difference between polygenes and major genes or oligogenes.

The difference is maintained by Mather (1949), who attributes to the polygenes small, similar and supplementary effects, and to the major genes such great action that—except in polyploids—the homozygous deficiency of one locus of each cannot be compensated by another. Mather gives an analogy of his idea in which major genes might be compared to the skeleton of the genome, the polygenes being their cloth. The Author, on the other hand, believes that between polygenes and oligogenes should exist transitional stages. In addition, Mather considers that pleiotropy is so far not proved for polygenes, although he does not exclude the possibility (Mather, 1949, p. 102).

Against this view, objections were raised by Robertson and Reeve (1952), pointing out that oligogenes have often a very slight effect, and that some alleles (iso-alleles) overlap. These remarks—we believe—seem based on a misunderstanding of Mather's thought, because the question does not concern the intensity of effect of alleles between them, nor the existence of small mutation steps. The point is that some genes (oligogenes) give rise to lethal genotypes in the case of their homozygous deficiency, while some genes (polygenes) do not, because other genes—duplicating their action—can replace those which are lacking. That such genes exist is actually proved by several sets of evidence. One is provided by the Y chromosome of *D. melanogaster* where only a few oligogenes are located, together with other multiple factors or polygenes, according to Mather and to the data shown in the present paper. These factors—as many investigations have shown for the Y chromosome—are unable to cause lethality when deficiencies occur.

The present paper, thus, supports the view that oligogenes and polygenes collaborate to determine a character (the cell size). The difference between two types of genes (which must obviously be considered connected by intermediate types) appears in the case in question by no means as qualitative, but purely as a quantitative one, the oligogene m-m^{+} alone being capable of producing a considerable effect, which numerous polygenes cannot equal.

One point in the polygene theory is considered by Mather, but largely elaborated by Robertson and Reeve (1952) and even more generally by Grüneberg (1952); that oligenes might have secondary effects, detectable as polygenic. This remark, tending obviously to eliminate any difference between oligogenes and polygenes cannot be ignored. It is possible that in some cases it may be true. Nonetheless, the Y chromosome of *D. melanogaster* provides an example of a system of genes, where a prominent polygenic activity exists.

(c) *Sources of genotypic variation in cell size.*—The foregoing discussion leads to a consideration of cell size as changing under genic control.

In order to see as clearly as possible the changes occurring in the case involved, we must consider a population of flies, where mutation and crossing-over act constantly. Owing to the rarity of mutation for single loci, changes in cell size, as big as those brought about by a mutation step of oligenes, like from m^+ to m^2 or *vice versa*, will contribute but little (or after long selection processes of casual fixation of genes) to the genotypic variation of the population. On the other hand, the mutability of a high number of polygenes scattered throughout the genome, including the Y chromosome, can provide a rather large number of new genotypes, producing very small, but relatively frequent changes in cell size.

The question now arises of the selective significance of the cell size. The cell size, actually, must be considered as correlated with the organ size, where the relationship between cell and organ size is mainly represented by the number of cells. When this number remains constant, every variation in cell size results in a parallel variation in the organ size. When the cell number decreases on the other hand, the cell size increase can also be counteracted, and the organ can even diminish as a whole. One secondary factor in determining the organ size is represented by the intercellular substances, which in certain tissues can play an important role; this is not the case for the wing.

For the wing of *D. melanogaster* we know after the investigations by Robertson and Reeve (1952), that not only the wing surface, but also the thorax length depends on cell size. We can thus imagine, how the continuous variation of cell size produced by polygenic action, can be intensively modified by selection.

Apart from the case of *D. melanogaster*, it will be worth investigating other species where big Y chromosomes are present (as in some Rodentia), whether a similar complex determination of cell size is at work.

5. SUMMARY

1. Cell size was analysed both on the wing and on the corneæ of *D. melanogaster*, with the aim of studying its genetic determination.

2. Three groups of genes have been found acting on this character:

(i) the pair of allelomorphs (m^+-m^2) located at 36·1, I.

(ii) many other genes located throughout the genome, which have not been analysed in detail.

(iii) many genes acting as polygenes, located in the Y chromosome.

3. The alleles m^+-m^2 act as major genes, and in some of their manifestations are modified by the Y chromosome.

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THE FREQUENCY OF TASTE-BLINDNESS IN WELSH POPULATIONS

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1. INTRODUCTION

The ability to taste certain solutions of phenyl-thio-carbamide in water is sharply differentiated in the human race. This is illustrated by Fisher and Falconer (1947), who present data accumulated over several years at the Galton Laboratory, London. An unequally peaked U-type frequency-histogram is commonly found when the results of any survey are graphed.

It is believed that the ability to taste and not to taste the solution of PTC is caused by a simple single autosomal gene-substitution, T and t. T is believed to be a normal dominant to the recessive, non-taster gene, t, therefore the homozygous-dominant, (TT), and the heterozygote (Tt), would result in an individual capable of tasting very high dilutions of PTC, whilst only the homozygous-recessive (tt), would result in a non-tasting individual. It has been suggested that this dominance may be incomplete, especially in the male. The various conditions are polymorphic in the human race, very approximately, one person in four being a non-taster in European and white-American populations. Possible differences may, however, exist between one population and another where some form of isolation, partial or complete, is in force between them. The present research was undertaken to investigate the existence or otherwise of such a possible difference between the general Welsh population and the population of the mountain-moorland of Plynlymon in Wales.

2. METHOD

An objective method of correctly determining the threshold value of an individual, with the minimum experimental error, requires some ingenuity. The principle used in the present work was largely that described by Fisher and Falconer. A logarithmic series of dilutions of PTC in water were prepared, from a saturated solution of 1600 parts of PTC in a million parts of water to extremely high dilutions of the order of $3\frac{1}{8}$ parts in a million. The process employed consisted of first manufacturing the 1600 solution by dissolving the PTC in heated tap-water (it was decided to use tap-water in this instance as the local water used is extremely free from dissolved mineral salts) and then diluting by half for each other solution.

When a solution is found that is sufficiently concentrated for a taste to be apparent, the solution of PTC will have a bitter taste.

Increasingly concentrated solutions of PTC, from the $3\frac{1}{8}$ parts in a million to the 1600 parts in a million (for brevity, these will henceforth be referred to as the $3\frac{1}{8}$ and 1600 solutions, with similar abbreviations for the other concentrations) were given to the subject until a definite taste had been detected. Unless a very strong taste had been noticed in the threshold solution the next strength of solution was given to the subject, increasing any detectable taste already felt, as an insurance that the correct threshold had in fact been found. This process enabled a first approximation to the correct threshold to be obtained.

Harris and Kalmus have recently suggested a refinement on the previously used simple determination of threshold. This addition consists of presenting the subject with eight beakers, randomly arranged, four of which contain the threshold solution, four plain water. If these are correctly separated, the experiment is repeated with a solution of greater dilution, until the subject can no longer correctly separate the beakers ; this is then assumed to be the true threshold. It was thought that whilst this procedure is theoretically correct, some experimental difficulties might be encountered in practice that would invalidate the precision of the method. Any impurity, or staleness of the PTC due to storage, would allow a false threshold to be determined. To obviate this difficulty an alternative arrangement was used in the determinations given in this paper, wherein four of the eight beakers contained the threshold solution already roughly determined and the remaining four, the next higher dilution. This ensures as far as possible that the differentiation shall be one of taste for PTC alone.

That differences do occur between the two methods may be seen from table 1 showing double-determinations on some taster and non-taster subjects.

TABLE 1

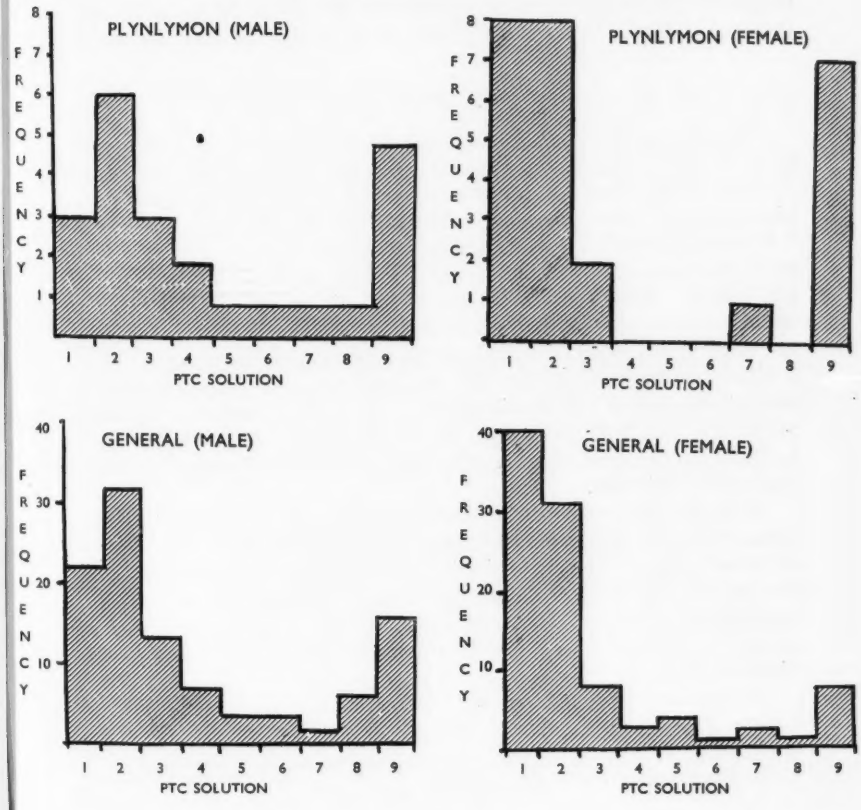
Method \ Threshold	♀				♂						
	A	B	C	D	E	F	G	H	I	J	K
Harris and Kalmus .	$\frac{3}{32}$	$3\frac{1}{8}$	$6\frac{1}{4}$	50	$3\frac{1}{8}$	$6\frac{1}{4}$	$6\frac{1}{4}$	$12\frac{1}{2}$	50	400	800
Beach . . .	$1\frac{9}{16}$	$6\frac{1}{4}$	$6\frac{1}{4}$	400	$3\frac{1}{8}$	$6\frac{1}{4}$	$12\frac{1}{2}$	50	800	1600	1600

It can be seen that in the cases of non-tasters, of both sexes, some appreciable variation is apparent. In some of these estimations of threshold the separation by the Harris and Kalmus method was made without the subject being able to state that a definite taste was present in the PTC solution, other than that it had a " stronger " taste than water. As the taste of PTC never becomes very pronounced in the definite non-taster, any foreign taste present might easily be mistaken for a definite taste, resulting in the correct separation of

the beakers. This error was made almost completely by non-tasters (*i.e.* subjects who could not detect a distinct taste in the rough test until solutions of the order of, at least, 400 were reached). The PTC solution in the Harris-Kalmus randomisation was then referred to as "stale", "strong", or as a "chemical solution", even far below their previously determined threshold. It is believed that this may be due to either the almost inevitable slight impurity of the PTC or to a side-effect of the PTC itself, only detectable against pure water solutions. When this effect is minimised, as with the randomisation used above, far more accurate results are obtained.

3. SAMPLING

In the general Welsh sample, the subjects tested came from all parts of Wales and were of Welsh ancestry. This latter condition was considered satisfied if both parents were born within the boundaries of Wales. The Plynlymon Moorland sample contained people with



restricted local ancestry only ; sometimes it was noted that all eight great grand-parents were born within the limits of the locality.

Both general and Plynlymon samples were collected during the summer of 1952. For the general Welsh sample, various South and North Wales' towns were visited and places of sampling selected at random ; subjects were then selected in a similar manner. It was found that the subjects tested came from all parts of Wales. No particular profession, class or type, sex or age-group was chosen and once a subject had been approached every possible effort was made to include him (or her) in the sample. In this way, only four definite refusals were encountered, out of some 203 individuals actually included in the general sample and none at all in the Moorland sample.

Great care was taken to keep the sampling as random as possible. The day, time of day and place of sampling were all chosen by random-sampling methods, usually by suitable numbering of the possibilities and the use of Fisher and Yates' tables of random-numbers. If a house was visited, the choice of the inclusion of one person of a family in the sample was left to similar unbiased methods.

4. RESULTS

The taste of the threshold solution of PTC commonly found was quite different between the two groups of tasters and non-tasters. The taster described the sensations as similar to quinine, being very bitter indeed, or, almost unanimously in Welsh rural areas, as " Wermod Lwyd ".* The comment " Mae'n chwerw iawn ! " was often encountered.† The non-tasters varied considerably more than the tasters. Taste sensations among the non-tasters ranged from a definite bitter taste, at the threshold, through a mildly sour or sharp, not unpleasant, tang, to no taste at all in some extreme cases.

The results obtained in this research are now given, corrected for age, in table 2 and in histogram form. The samples are divided up by the nature of the sample (General and Moorland Welsh) and by the sex of the person tested. The number obtained for the thresholds shown are indicated.

It is customary to choose the threshold value that divides the tasters from the non-tasters as that which contains the least number of individuals. Using this criterion, the dividing thresholds become the rather high values of 200 for women and 400 for men. The number of tasters and non-tasters may then be arranged in an hierarchical classification and tested for homogeneity at each stage of the division (table 3).

It may be seen that neither of the multiple divisions, either of

* " Wermod Lwyd "—the herb wormwood. Solutions of this herb in water are drunk in Welsh country areas medicinally and for tonic effects, it is very bitter to the taste.

† " Mae'n chwerw iawn "—it is very bitter.

sample-locality or of sex, show significant heterogeneity. The value of χ^2, for the between-samples heterogeneity, however, is very near the borderline value of significance. The principal reason for this non-homogeneity appears to be the larger percentage of non-tasters

TABLE 2

Classification of samples by sex and locality of sampling

	Strength of PTC in solution									Totals
	Up to 6¼	12½	25	50	100	200	400	800	1600 and over	
Plynlymon (male) .	3	6	3	2	1	1	1	1	5	*23*
General (male) .	22	32	13	7	4	4	2	6	16	*106*
Plynlymon (female)	8	8	2	0	0	0	1	0	7	*26*
General (female) .	40	31	8	3	4	1	2	1	7	*97*
Totals . .	**73**	**77**	**26**	**12**	**9**	**6**	**6**	**8**	**35**	**252**

in the Plynlymon Moorland sample (44 against 29 per cent. in the latter). It is possible that larger samples, of both regions would either confirm or deny this heterogeneity more definitely. However, as the heterogeneity of the samples divided by sex is definitely not

TABLE 3

Hierarchical division of samples

	Individual samples		Male and female			Total	
	(*tt*)	*n*		(*tt*)	*n*	(*tt*)	*n*
Plynlymon (male) . .	7	23					
General (male) . .	24	106	(Male)	31	129	50	252
Plynlymon (female) .	8	26	(Female)	19	123		
General (female) . .	11	97					
χ^2			5·5877		2·9174		
Degrees of freedom . .			2		1		
Probability			0·06		0·09		

significant, the separate sex-divisions may be merged to give larger numbers to the test of heterogeneity of the place of sampling.

When this is done, the χ^2, with one degree of freedom, is 4·4371 which has a probability of between 0·05 and 0·02, values which may be considered significant.

5. DISCUSSION

The Moorland is a high bleak plateau some fifteen miles from the Welsh coast, with an altitude of from 800 to 1500 feet. The great majority of its population may only be reached by semi-surfaced or unsurfaced cartroads and are thus extremely isolated from the outside world. In addition, it is very highly probable that this isolation was far more complete up to comparatively recent times. Very little intermarriage occurs between the Welsh people of this mountain moorland and the people of the lowlands, consequently, by reason of its comparatively small area, the rate of consanguinity is high. One family was found in which first cousin marriages had occurred for the three previous generations. The people of the moorland seldom leave the area despite the extremely harsh conditions of life, whilst newcomers from the lowland areas seldom stay into the second generation. It is possible, on this account, that the genic-constitution of the people of the moorland might differ, in a statistically significant manner for some particular characters, from the genic-constitution of the Welsh of the lowlands. In this respect, there is some recent evidence of Mourant and Watkins in which the blood-group B-gene frequencies of the Black Mountains of Carmarthenshire, the Mynydd Hiraethog of West Denbighshire and the Plynlymon Moorland were far higher than in the surrounding countryside.

It is possible that these results and indeed some of the undoubted differences in expression of the taster-gene, may be explained by some environmental effects. Similar results have been found in animals and certain insects that indicate that such an hypothesis is, at least, possible in man. It is also possible, that the anomalies that do occur may be better explained by either an alternative gene, possibly an allele of the T, t allelomorphs, or by a modifying factor. Some few exceptions to the simple, single-gene substitution with dominance have been found in the earlier investigations of Snyder, where some " non-taster " × " non-taster " matings resulted in " taster " children. It is perhaps most likely, however, that these exceptions are either physiological or the result of incomplete expression of the gene.

In this paper, some tentative division into tasters and non-tasters by the usually accepted means of minimal frequency has been adopted. This has been done mostly for comparison purposes. However, it will be apparent that genotypic assumptions from this division are to some unknown extent, arbitrary. With this qualification, the gene-frequencies of the general Welsh population and Plynlymon Moorland Welsh populations for the recessive t-gene are 0·4152 (±0·0638) and 0·5533 (±0·1190) respectively.

In all events, when no account is taken of sex, some significant differentiation is apparent between the Plynlymon Moorland population and that of the general Welsh population for the tasting-ability.

It is probable, with the above-mentioned reservations, that this differentiation is genetic in character.

6. SUMMARY

1. A modified randomisation test for accurate determination of the threshold level of taste for PTC is described.

2. In tests for heterogeneity between the main Welsh population and the population of the Plynlymon Moorland, some non-significant heterogeneity is found between the separate samples but none between the simple divisions by sex.

3. On amalgamation, without regard to sex, significant heterogeneity is found between the two main samples. Their gene-frequencies were 0·4152 (±0·0638) and 0·5533 (±0·1190) respectively.

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QUANTITATIVE VARIATION IN SUBSEXUAL RUBUS

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I. SUBSEXUAL REPRODUCTION

In apomictic lines the occurrence of individuals not resembling the mother was attributed, in the early days of genetics, to mutation, as shown in the works of Lidforss and Ostenfeld. Yet segregation and recombination can follow from crossing-over when reduction is suppressed. How this situation arises with diploid parthenocarpy in plants has been explained by Darlington (1932), who later (1937) named it subsexual reproduction (table 1). Gustafsson (1942) drew attention to the significance of such "auto-segregation," especially with regard to the European blackberry flora.

TABLE 1

Permanent modes of reproduction

Vegetative reproduction (No meiosis : nucellar embryony, etc.)	*Diploid parthenogenesis* (Suppressed meiosis, no reduction, no fertilisation)	*Sexual Reproduction* (Reduction and fertilisation; recombination also by crossing-over)

True-breeding (No crossing-over or chiasmata)	*Subsexual reproduction* (Auto-segregation and recombination resulting from crossing-over and failure of first or second division)

Since Lidforss in 1907 showed evidence of auto-segregation in Rubus, further study was not made until Darrow and Waldo (1933) obtained auto-segregation in parthenogenetic Rubus. In Rubus species-crosses Crane and Thomas (1939, 1940) also showed the presence of true sexual hybrids along with maternal, that is parthenogenetic, offspring which themselves varied. Gustafsson (1942) explained that this is important as a means of biotype formation in apomicts with extinct sexuality.

Previous observations of Lidforss, Ostenfeld and Gustafsson were concerned with qualitative variations. The only attempt to study quantitative variation has been that of Babcock and Stebbins (1938), who were interested in showing greater variation among sexual than among apomictic forms of Crepis and seem to have assumed that the apomicts were constant. This they did by comparison of inflorescence

and bract sizes in apomictic and sexual strains. In the light of what I am going to show, they were studying the first example of quantitative variation with subsexual reproduction.

In all previous instances with Rubus, auto segregates were produced morphologically distinct from the mother plant. In the present study two families totalling 309 plants of Merton Early blackberry were investigated. But no plant could clearly be said to differ sharply from the rest. Only continuously varying quantitative characters were therefore available for study.

2. MATERIAL : *RUBUS NITIDIOIDES*

Rubus nitidioides Watson, is a cultivated tetraploid blackberry ($2n = 28$), known as Merton Early, sometimes clonally propagated from stem tips but more often grown from seeds. There is little variation in these seedlings (Crane, 1941). Yet although recognising it as apomictic, Crane and Thomas (1939) observed some variation in apomictic offspring after pollination with *R. thyrsiger* ($2n = 28$). They concluded that apomictic embryos arose by subsexual reproduction. In detailed cytological and embryological studies, Thomas (1940) found that *R. nitidioides* reproduces mainly by apospory, which does not allow for segregation, and also by the fusion of two haploid nuclei within the embryo-sac, which would allow for segregation.

The variation found by Crane and Thomas was largely quantitative. In the present biometrical analysis I have therefore attempted to present some of the features of quantitative variation in offspring of " selfed " and " sibbed " families of subsexual *R. nitidioides*, in relation to the theory of auto-segregation. Assessment is then made of the possible value of auto-segregation in plant improvement and the possibility discussed of effective selection within subsexual families.

3. METHODS

Seeds of a number of closely similar open-pollinated plants were collected and sown together in seed-boxes at Merton in 1945. Plants from these are Family 31/45. Flowers of one plant (No. 1) having good appearance were selfed, and their seeds sown as Family 32/45. Seedlings were transferred to permanent rows at Bayfordbury in 1946 and spaced 2 ft. apart in rows. As Rubus germinates irregularly, three separate plantings of both families were made. The results of all plantings have been pooled for analysis. Plants were strung up with not more than four shoots per plant, and old branches annually cut back.

Altogether 84 seedlings of Fam. 31 and 225 of Fam. 32 were planted out. Some plants have suffered from depression, due to an unidentified disease. Quantitative measurements were taken in 1952. Prickles were counted on the two internodes of each available branch

at 3 ft. from the ground, and mean prickle number per internode calculated. Prickle size was judged by the eye on internodes at $5\frac{1}{2}$ ft. from the ground, or towards the ends of branches when these were lower. In addition, records were taken of times of first flowering, and of first fruit maturity. Qualitative characters such as prickle type, flower colour, leaflet number, and whether inflorescences were long or short, were also noted. All these were non-segregating, both families being remarkably uniform morphologically. There were no noticeable differences in fruit flavour.

4. RESULTS

(i) *Plant vigour and shooting ability*

Plants were placed in five categories for vigour, measured in April-May 1952. Growth ranged from very weak types, obviously attacked by a fungus or virus, to highly vigorous plants. The histograms in fig. 1 show that most plants of both populations are vigorous (v).

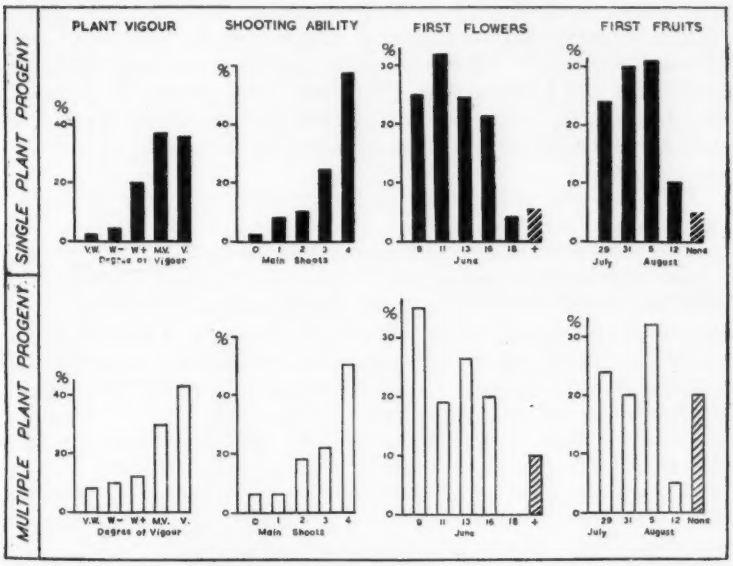

FIG. 1.—Histograms of quantitative characters in *Rubus nitidioides*. Offspring from several "open-pollinated" plants (Fam. 31) represented by open bars; offspring from one selected plant "self-pollinated" (Fam. 32) by black bars.

Reduced vigour of very weak (v.w.) and some of the weak (w—) ones is directly attributable to disease depression. Fam. 31 differs slightly from Fam. 32 as it contains more plants in the extreme categories, while in Fam. 32 there are comparatively more plants in the central part

of the distribution range. The more extreme differences in Fam. 31 in vigour could be due to the tails of several distributions overlapping. Fam. 31 might therefore consist of the frequency distributions of separate apomictic auto-segregating populations.

Shooting ability in spring was judged by the number of shoots tied up in autumn ready for flowering the following year. The maximum was four, if available. Fig. 1 shows that somewhat more plants having more shoots were produced in Fam. 32. The parent of Fam. 32 had been selected for vigour and general good plant characters; its progeny show this influence slightly. The plants were no less vigorous than those of a clonally reproduced stock. Inbreeding depression is thus not present. There is a positive correlation between shooting ability and plant vigour, not unexpected, as one is an index of the other. Hence the more vigorous seedlings are likely to produce plants with the more abundant fruiting.

(ii) *Prickle size and number*

Histograms for prickle sizes in both families show that slightly more plants in Fam. 32 have larger prickles. Data on very small prickles are somewhat unreliable, as disease infection reduces prickle size and frequency. There is a strong positive correlation between prickle size and plant vigour. The latter was greater in Fam. 32 than in Fam. 31, and this may account for the higher frequency of larger sized prickles in Fam. 32. All prickles were of uniform morphology.

Frequency distributions for prickle numbers are given in fig. 3. Here it is seen that Fam. 32 has a normal frequency distribution with a sharp peak between 20 to 24 prickles per internode. Fam. 31 has a somewhat narrower range and no sharp peak, the frequency from 16 to 24 prickles per internode being the same, and with a slight increase only from 24 to 28. It is clear from fig. 3 that Fam. 32 comprises a single segregating population, while Fam. 31 contains a series of overlapping frequency distributions with peaks both higher and lower than 20 to 24, as in Fam. 32.

Actual number of prickles, prickle size and plant vigour are also correlated, although average prickle number only increases by two prickles between plants of weakish habit and very vigorous ones (fig. 2). These correlations have to be borne in mind when attempting to select less prickly but vigorous plants for breeding purposes.

(iii) *Blossoming and fruiting*

Histograms of the frequencies of first flowering times are given in fig. 1. Here it is seen that Fam. 31 contains more at the extreme periods of flowering, although the range of Fam. 32 is just as wide. Over 90 per cent. of both populations had flowered within nine days. Considering the effects due to pruning and other environmental factors, both populations are remarkably uniform in flowering.

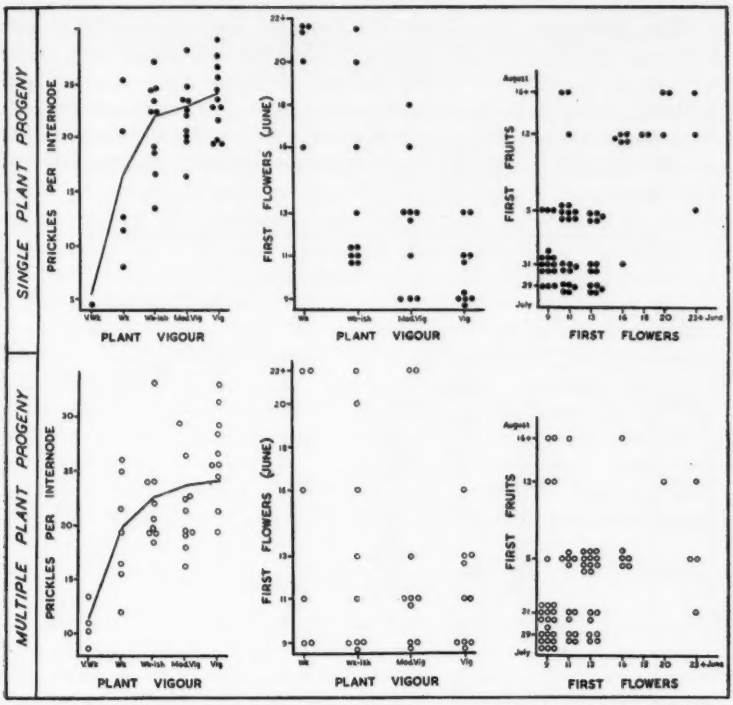

FIG. 2.—Correlations between quantitative characters in two families of *Rubus nitidioides*. Open circles represent Fam. 31 from several "open-pollinated" plants, and black circles Fam. 32, from one selected plant "self-pollinated".

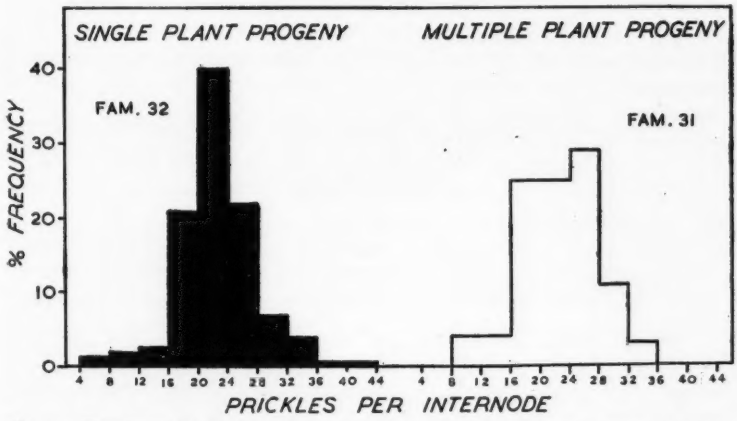

FIG. 3.—Frequency distributions of mean prickle number per internode, grouped in units of four, in two populations of *Rubus nitidioides*. Left histogram represents Fam. 32, from one plant "selfed"; right histogram represents Fam. 31 from an "open-pollinated" population.

The percentage frequencies for times of appearance of first ripe fruits are modified by those plants in both families which were either too late in fruiting, or lacked flowers, or these were diseased and died off. Little difference can be detected in this character between the two families; they are remarkably alike (fig. 1), except that 20 per cent. of Fam. 31 failed to fruit, almost entirely on plants affected by disease.

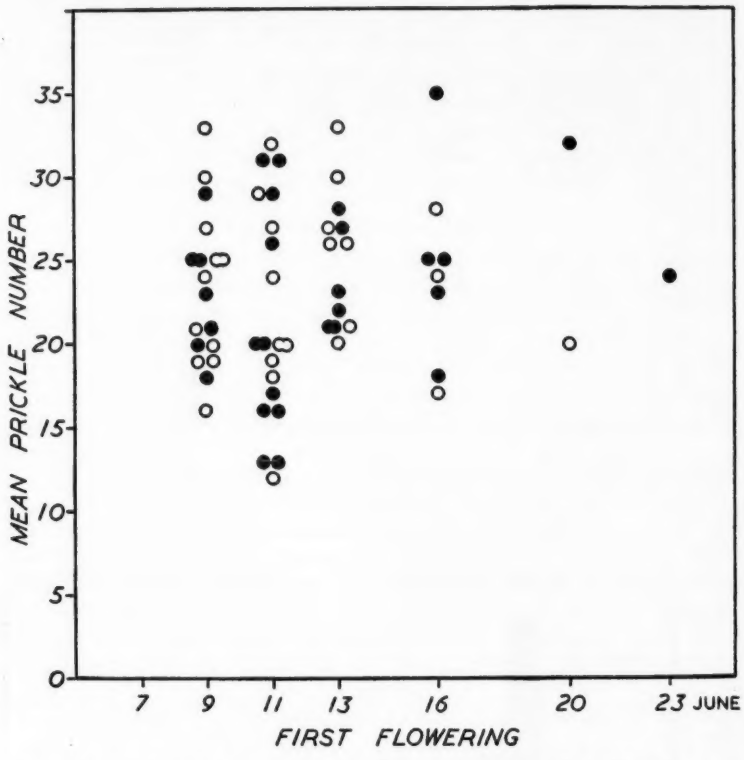

FIG. 4.—Scatter diagram to show range of variation with respect to prickle number per internode and to first flowering times within two populations of *Rubus nitidioides*. Fam. 31 (from open-pollinated parents) is represented by open circles; Fam. 32 (from one selfed plant) is represented by black circles.

Fig. 2 shows the correlation between first flowering and first fruiting, and indicates that for these characters it is surprisingly weak. Plants with earliest flowers do not necessarily produce the first ripe berries. Many environmental factors must come into play to effect fruiting. There is also moderate correlation between first flowering and general plant vigour.

5. VARIATION RANGES IN PURE AND MIXED APOMICTIC FAMILIES

A test was made to compare the range of variation in both families. This helps to determine how far variation differences between families are due purely to variation within an apomict or, a possibility in Fam. 31, to sexual segregation. Two quantitative characters, considered sufficiently unrelated, were used. These were prickle number and time of first flowering. Data were taken from thirty representative plants and plotted as scatter diagrams (fig. 4), following the method of Babcock and Stebbins (1938).

Distribution and concentration for both families is remarkably similar. Thus although slightly wider segregation of prickle number was discerned in Fam. 32 (*cf.* fig. 3), there is no indication that it is of the degree expected with sexual segregation. Similarly, the multiple progeny of Fam. 31 showed no wider variation range than that of the single progeny in Fam. 32. Thus although variation is detectable within different families of *R. nitidioides* according to their ancestry, it is extremely limited and is in the nature of subsexual auto-segregation.

6. ORIGIN OF SEGREGATION

The highest prickled plant, called H_1, showed several associated quantitative features such as late flowering, comparative paucity of inflorescences and fruits, and its drupels often swelled without seed formation, *i.e.* by parthenocarpy. On the other hand, the next highest plant, H_2, and the two plants with lowest prickles, called L_1 and L_2 respectively, were similar to others in both populations. It cannot therefore be ruled out that H_1 results from recombination following true sexual union ; nor is there any way of detecting true sexual plants among apomicts within the families. In the open-pollinated Fam. 31 there is the possibility of their occurrence and recognition in chance outcrosses with other species. However, there were none, nor was any major gene segregating. A character such as anthocyanin would be extremely useful as a marker gene in these studies : on the basis of Vavilov's law of homologous variation, it may eventually be obtained as a mutant because it occurs in other Rubus species.

It is not necessary to attribute any of the quantitative variation to loss or gain of chromosomes, for Thomas (1940) showed that chromosome number is constant in *R. nitidioides*. Moreover, since no major differences were seen, monosomics such as found in variants of apomictic *Erigeron annuum* and Taraxacum (*cf.* Gustafsson, 1947) need not be suspected.

As between the families, 31 was more variable in some characters, but both were remarkably similar for most such as time of first fruiting. On the whole, greatly restricted variation is present, well shown by the scatter diagrams of the variation ranges (fig. 4). Yet there is still

sufficient subsexual variation to allow detection of differences according to the genetic history of each family.

Morphological variation in apomicts besides Rubus are known, *e.g.* in *Parthenium argentatum* (Rollins, 1945) and Taraxacum. In Parthenium variants occur not only with reduced chromosome number, shorter trichomes and pollen differences, unlike Rubus, but also among individuals showing no chromosomal changes ; these deviations within the apomicts have slower growth. Because environment was constant, Rollins attributed this latter variation to genetic differences within the same age-group. As rather small families were used, possibly a continuous variation as in *R. nitidioides*, rather than the assumed discontinuous differences, also occurred in Parthenium.

It may be asked whether a plant has greater chances of true sexual reproduction when selfed than when sibbed (*i.e.* sister-crossed) or outcrossed to other species. Gustafsson (1939) assessed available evidence in Rubus and found that the amount of sexual fusion is unrelated to parental relationships. Thus although some of the variation might be attributable to the pollen parent, probably this has not been of importance in influencing the variations of either Fam. 31 or 32. And only the seed-bearing plant, where crossing-over occurs, is of practical interest. One may conclude that because of difficulty in clonally propagating Merton Early, it may safely be raised from " selfed " seed of best plants without deterioration or immediate deviation in the stock, either from inbreeding depression or from subsexual segregation.

7. CONSEQUENCES OF POLYGENIC VARIATION

Mather (1949) has described the obscuring effect of non-heritable variation on the segregation of polygenes in sexual plants. Even though prickle number per internode was highly variable within plants, the effects based on mean values are quite clear (fig. 3). Non-heritable variation no doubt plays a large role in influencing the quantitative variation of apomicts like the progenies of Merton Early ; even greater within-plant variation might be expected when they are grown under unsuitable conditions to which they are unadapted (Haskell, 1953). Since groups of polygenes acting as effective factors are subject to mutation and recombination, one can predict that following quantitative selection, even subsexual apomicts will ultimately show correlated responses like those of sexual species, *e.g.* Drosophila (Mather and Harrison, 1949) and various plants (Haskell, 1951). Thus from the current experiment on selection for high and low prickles we shall be able to see whether, for example, there is a gradual drop in fertility. Similarly, if as Crane (1941) has suggested, *R. nitidioides* originates from hybridisation together with unreduced gametes, its heterozygosity is concealed by the resulting apomixis, yet auto-segregation is then facilitated (Gustafsson, 1947). Thus

heterozygosity together with mutation potentially can produce considerable differentiation. Hence a study of quantitative characters in *R. calvatus*, an apomict without segregation according to Thomas (1940), would be of comparative interest.

8. PRACTICAL BREEDING PROBLEMS

In Merton Early there are no complications due to segregation of chromosome numbers or unreduced gametes ; also there is only one morphological form. Hence at first glance there seems no direct disadvantage to the grower as to what seed he harvests, providing he rogues any sexual species-hybrids, easily recognisable among offspring of open-pollinated seed. Now there is reason to suspect that loganberry stocks have changed owing to very similar seedlings, resulting from self-sown seeds, being unwittingly selected. As such small segregations even in a more or less constant species-hybrid are eventually felt, selection of apomictic seed from vigorous early Merton Early plants with low prickles should eventually produce some recognisable advantageous mild selection. Such an advantage in an apomict should be utilised, however slight.

There was no inbreeding depression ; progenies from both " selfed " and mixed " selfed-sibbed " families had similar vigour. Whether the 5 per cent. true intra-specific sexual individuals, possible according to Thomas (1940), show inbreeding depression cannot immediately be determined. Even so, apomictic seedlings from selfings of the best plants will maintain the line's vigour. Lewis (unpublished) has found in raspberries that the first selfed generation is often more vigorous than the parents, owing to the elimination of viruses which are not seed transmitted ; but later generations show the true extent of inbreeding depression. So far, however, virus diseases are not known in Merton Early so this complication will not arise. Hence reproduction by seed is satisfactory, little variation in vigour either way from the original clone being expected. At the same time, owing to auto-segregation, seed should be retained only from earliest and most vigorous plants free from disease.

In practical cultivation there may be advantages in having some discontinuity in a sample of seedlings brought about by auto-segregation, for under cultivation complete uniformity of crops is not altogether desirable. Thus Hanson *et al.* (1952) have found mixtures of apomictic strains of *Poa pratensis* better yielding than single strains, especially when tall and dwarf types are mixed.

9. SUMMARY

1. Quantitative characters were studied in two pseudogamous subsexual families of *Rubus nitidioides*. One came from a single selected self-pollinated plant (Fam. 32) and the other from several open-pollinated plants (Fam. 31). Fam. 32 suffered no inbreeding depression following " selfing ".

2. Plant vigour histograms showed that Fam. 31, but not Fam. 32, might consist of several overlapping frequency distributions. Prickle frequency was normally distributed in Fam. 32, but Fam. 31 had no central peak, confirming that the former was a single, segregating population while Fam. 31 comprised closely overlapping segregations.

3. First flowering times and fruit maturities were highly uniform and similar in both families, but there was weak correlation for individual plants.

4. Variation ranges in both families were compared by plotting prickle numbers against flowering times. As both had similar distribution, there was no evidence of genuine and widespread sexual reproduction. Variation, although detectable, was extremely limited, attributable to auto-segregation rather than to true sexual segregation.

5. Prickle number might be reduced by selection. Hence further study of selection in subsexual apomicts has theoretical and economic interest.

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POLLEN FORMATION IN PENTAPLOID AND NEAR-PENTAPLOID WHEAT HYBRIDS

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1. INTRODUCTION

INTERSPECIFIC wheat crosses have been investigated cytologically at various times throughout the last few years. Interest has fluctuated with the importance of the hybridising work and the success with which disease resistance has been introduced from the tetraploid species, $4x = 28$, into the hexaploid wheats, $6x = 42$. The present study was stimulated by a new technique for examining the chromosomes at the mitoses in the pollen grains. Meiotic studies were also made because of the doubt cast on the validity of some of the earlier reports (Love, 1941). At the same time, material on hand made it possible to compare the behaviour in near-pentaploid plants and in pentaploid plants.

2. METHODS AND MATERIALS

All P.M.C's. were examined from squash preparations stained in aceto-carmine. Root-tips were pre-treated with monobromonaphthalene, fixed in 2 BD and then prepared as Feulgen squashes. Mitoses in pollen grains were examined using the method outlined previously (Morrison, 1953).

The following F_1 material was examined in detail :—

T. vavilovi × *T. dicoccum*	Plant C $5x$
T. vulgare × *T. dicoccum* :—	
H-44-24 × Khapli	Plant A $5x$
Chinese Spring * × Vernal . . .	Plant B $5x$
,, ,, . . .	Plant D $5x-1$
,, ,, . . .	Plant E $5x+1$

* Trisomic ($2n = 43$)

3. METAPHASE I

In all pentaploids and near-pentaploids no more than 14 bivalents per nucleus were formed (table 1). Pairing varied among the P.M.C's., with from 9 to 14 bivalents per cell within the same anther (figs. 3A and B). This variation contradicts previous reports that the pentaploid regularly forms $14^{II}\ 7^{I}$ at meiosis (Kihara, 1938 ; Aase, 1935, and Thompson, 1934, among others).

If the missing chromosome in plant D has a homologue in the

set of *T. dicoccum*, then the maximum pairing expected will be only 13 bivalents (table 1). The seven from *T. vulgare* plus the unmated *dicoccum*-chromosome will give 8 univalents. The extra chromosome in the parental trisomic was therefore one of the first 14 chromosomes (A and B genomes of Kihara). Trivalents are therefore expected in plant E and in the majority of P.M.C's.—$1^{111}\ 13^{11}\ 7^{1}$. Chain trivalents were formed from two chiasmata ; the rod-ring type (Mather, 1935) from three.

TABLE 1

Comparison of the pairing behaviour in pentaploid and near-pentaploid wheat hybrids. Univalents are not indicated

$5x$				$5x-1$		$5x+1$	
MI arrangement	Plant A (in per cent.)	Plant B (in per cent.)	Plant C (in per cent.)	MI arrangement	Plant D (in per cent.)	MI pairing	Plant E (in per cent.)
14^{11}	31·1	67·0	78·0	13^{11}	44·0	14^{11}	20·0
13^{11}	36·7	28·0	18·0	12^{11}	39·0	$13^{11}\ 1^{111}$	46·0
12^{11}	17·8	4·0	4·0	11^{11}	11·0	13^{11}	10·0
11^{11}	11·1	1·0	...	10^{11}	3·0	$12^{11}\ 1^{111}$	14·0
10^{11}	2·2	...	...	9^{11}	2·0	12^{11}	10·0
9^{11}	1·1	...	...	7^{11}	1·0	...	...
No. of P.M.C's. examined	90	100	50		100		50
Mean no. of bivalents per P.M.C.	**12·8**	**13·6**	**13·7**		**12·1**		**13·6***

* Trivalents calculated as bivalents.

At diplotene-diakinesis, in some cells it was difficult to distinguish between bivalents and univalents because of grouping around the nucleolus. However, in all cells with well-spread chromosomes there were 14 pairs, associated no doubt sometimes only by coiling. Horton (1936) also observed 14 pairs in pre-metaphase stages. The variation in pairing at MI is characteristic of all hybrids and is a result of the variability in chiasma formation following interrupted pachytene pairing. Some of the reduction in chiasmata may also be attributed to environment (Mather, 1935, and Li *et al.*, 1945).

Love (1941) found similar variation in the pairing behaviour in pentaploids. In one hybrid of *T. vulgare* × *T. durum* the pairing arrangement $14^{11}\ 7^{1}$ was only present in 5 per cent. of the cells. Love also found trivalents and higher associations. Except for two doubtful trivalents in plant B, I found no higher associations in the 35-chromosome plants.

4. ANAPHASE I AND TELOPHASE I

At AI and TI my results again disagree with previous results. Some of the univalents split equationally in the first division and are distributed at random in the second. But all of them do not. In plant C in a squash of three anthers, there were 60 cells at AI where the distribution at the poles could be counted (table 2). In B, the

TABLE 2

Frequency of chromosome distribution to the poles and the number of laggards remaining between them at AI, TI and TII

No. of laggards lying between the poles	AI			TI	TII
	Distribution of chromosomes to the poles	No. of cells with laggards	Per cent. of cells with laggards	Per cent. of cells with laggards	Per cent. of cells with laggards
0	17 : 18	1	1·7	...	...
1	17 : 17	1	3·3	2·5	3·8
	16 : 18	1			
2	...	...	...	10·0	8·8
3	16 : 16	2	10·0	15·0	19·5
	15 : 17	3			
	13 : 19	1			
4	15 : 16	7	16·7	12·5	19·5
	14 : 17	3			
5	15 : 15	8	18·3	25·0	23·9
	14 : 16	3			
6	14 : 15	17	28·3	17·5	15·7
7	14 : 14	11	18·3	10·0	6·9
8	13 : 14	1	1·7	5·0	1·9
9	12 : 14	1	1·7	2·5	...
Total no. of cells examined . .		60	...	40	159
Mean no. of laggards per cell .		5·2	...	4·8	4·4

distribution was varied as well and only one-sixth of the P.M.C's. at AI had seven laggards. In over 80 per cent. of the cells one or more of the univalents were included intact with the bivalents when they passed to the poles. This random distribution will give dyad nuclei containing from 12 to 19 chromosomes plus a varying number of divided univalents.

At TI the chromosomes that are crowded at the poles cannot be counted but the lagging univalents are clear. The mean of 4·8 univalents (table 2) again shows that 7 univalents were not equationally divided at AI. Univalents were split in all cells but in 10 per cent. of the cells both halves of one or more univalents were passing to the one pole.

In 4·3 per cent. of the P.M.C's. there was misdivision of the centromeres in one or more lagging univalents.

5. SECOND DIVISION

Interphase.—Lagging univalents or daughter univalents excluded from the TI nuclei form micronuclei (table 3). The difference in plant B may be due to chance or it may also be a reflection of the better pairing in this plant than in D. The sizes of the micronuclei vary considerably. Most frequently each one is an excluded daughter univalent.

Anaphase II and telophase II.—Sufficient cells could not be scored to give a comparison of the number of chromosomes at each pole at AII but at TII laggards are clear. The results given in table 2 refer to only half-tetrads. They were scored in this manner because of the lack of synchronisation in the two halves. While the results are again taken from plant C, for a comparison with earlier stages, laggards were observed in all pentaploids and near-pentaploids.

There was 10 per cent. misdivision at TII. This is probably an under-estimate because in some cells the centromeres were stretched by movement forces. Such cells were not scored as having misdivision but a break is more probable than inclusion of two separated arms.

In the earliest reports, a random distribution of the 7 chromatids at second division was assumed. Later, Kihara and Matsumura (1940) suggested that the univalents went in groups to the poles. There is no evidence in my material for such a grouping at AII or at TII.

Tetrads.—Most of the tetrads, like the dyads, had one or more micronuclei (table 3). Here I agree with Love (1941). Microcytes were formed in less than one per cent. of the tetrads. It is evident from the large number of micronuclei both in pentaploids and near-pentaploids, that there is considerable chromosome loss.

TABLE 3

Frequency of micronuclei formed in dyads and tetrads from excluded laggards arising in pentaploid and near-pentaploid hybrids

Plant and chromosome number	Dyads				Tetrads			
	No. of cells examined	Per cent. with micronuclei	Micronuclei per dyad Range	Mean	No. of cells examined	Per cent. with micronuclei	Micronuclei per tetrad Range	Mean
A ($2n = 35$)	277	93·5	1-9	3·5	348	99·4	1-9	4·0
B ($2n = 35$)	457	78·3	1-5	1·6	250	99·6	1-9	3·7
C ($2n = 35$)	...	...	...		356	98·6	1-8	3·0
D ($2n = 34$)	203	96·6	1-7	3·7	524	98·7	1-10	4·4
E ($2n = 36$)	229	90·0	1-6	2·4	279	96·1	1-6	2·3

6. POLLEN GRAINS

The micronuclei formed in the tetrads persist in the pollen grains until the cytoplasm becomes vacuolated and lines the wall. Then they are absorbed, presumably by the cytoplasm. At first mitosis less than 5 per cent. of the P.G's. had micronuclei ; these were mostly inactive.

At the time of first mitosis, while there was some variation in P.G. size there was no correlation between size and number of chromosomes in the P.G's., *e.g.* those grains with 14 were similar in size to those with 21. Approximately 1 per cent. of micro-pollen grains were formed from microcytes. After 1st mitosis the grains become filled with starch and the cytoplasm becomes more dense. Differential growth rates due to unbalanced P.G's. could thus arise after 1st mitosis.

P.G's. with numbers from 14-21 were present in anthers which varied in age (as tested by the number of bi-nucleate and uni-nucleate grains present, table 4). This might mean that there is no differentiation with respect to entry into the first mitosis. However the number

TABLE 4

Distribution of the P.G's. with varying numbers undergoing mitosis, from anthers at different ages

Binucleate P.G's. in the anthers	Approx. no. of P.G's. in the squash	No. of P.G's. at metaphase	Range in chr. nos. of P.G's.	Mean chr. no.
37 per cent.	1000	19	15-20	17·4
40 ,,	1000	19	14-21	17·1
70 ,,	1000	31	15-20	17·4

of P.G's. actually undergoing division was usually less than 2 per cent. of the cells. Therefore the results may not be indicative of the true picture.

At first mitosis, there is no significant difference between the three pentaploids for the various classes from 14 to 21 (table 5). Even in plant D, which has a maximum of 13 pairs at MI, the distribution of the P.G's. does not deviate very much from the others.

The theoretical frequency (fig. 1) is based on the chance distribution of 7 univalents. The chief differences between the observed and theoretical are the depression of intermediate numbers and an excess of both 14 and 21 P.G's. There are several factors which have altered the proportion of the classes.

(i) Loss of univalents as micronuclei (table 3) reduces the chance that all 7 univalents will go to one pole. This should cause a reduction in the higher numbers.

(ii) Reduced pairing (table 1) gives more than 7 univalents. This should extend the range in chromosome numbers.

(iii) Random distribution of non-divided univalents at AI (table 2). This should cause an excess of intermediate numbers.

(iv) Competitive ability of balanced P.G's. This will bring about an equilibrium with the first three factors—more P.G's. with higher numbers ; fewer with a complement of less than 14 or more than 21 ; and fewer P.G's. with intermediate numbers.

TABLE 5

Distribution of the chromosome numbers at 1st and 2nd mitosis in the P.G's. of 5x and near-5x plants

Plant and chromosome number	Total no. of P.G's. examined	P.G's. with various numbers of chromosomes (in per cent.)								Mean chr. no. per P.G.‡
		14	15	16	17	18	19	20	21	P.G's. with ff	
A . . .	29	3·4	10·3	13·8	24·1	17·2	13·8	10·3	6·9	...	17·6
B . . .	182	4·9	11·0	11·5	19·8	19·2	15·9	9·3	4·9	3·3*	17·5
C . . .	100	1·0	12·0	15·0	22·0	30·0	12·0	4·0	2·0	2·0	17·3
Total (5x) .	311	3·5	11·3	12·9	20·9	22·5	14·5	7·7	4·1	2·6	17·5
D (5x−1) .	100	5·0	10·0	12·0	20·0	24·0	20·0	6·0	...	3·0	17·4
Generative cell division											
B . . .	35		2·9	17·1	20·0	37·1	17·1	2·9	...	2·9†	17·6

* Including two abnormal P.G's. one with 10 and one with 12 chromosomes.

† One abnormal P.G. with 32 chromosomes.

‡ Omitting fragments.

ff = fragments.

An examination at the second division should show whether unbalanced P.G's. with intermediate numbers suffer more in competition as they mature and become filled with starch. Unfortunately the division of the generative cell is difficult to study and the few counts (table 5) were made up from several slides. Therefore while it does mean that many intermediate-numbered P.G's. do form gametes, it does not show the proportions of each type that are undergoing division at any one time.

Mature pollen prior to anthesis showed : (i) size differences, (ii) aborted grains, approximately 10 per cent., (iii) some P.G's. in which division had failed, (iv) differences in the amount of starch in the grains. The aborted grains and those with reduced starch were probably due to unbalanced combinations.

My count of the chromosome number in P.G's. agrees closely with those of Thompson and Armstrong (1932) and Matsumura

(1936*a*). These workers did not record fragments. With the new technique fragments are shown clearly (fig. 3E). The fragments in the P.G's. arise from misdivision of univalents. Five of the nine fragments were telocentrics. In the other four the positions of the centromeres were doubtful. There is a smaller proportion of P.G's. with fragments than was observed in the monosomic wheats (Morrison, 1953*a*). This reduction agrees with the reduced misdivision frequency at telophase.

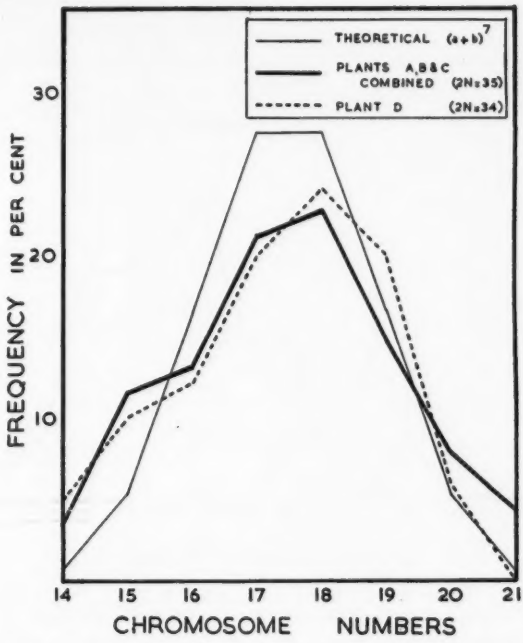

FIG. 1.—Frequency distribution of chromosome numbers in pollen grains in pentaploid and near-pentaploid plants.

The two hypoploid P.G's. (table 5) could result from either segregation after reduced pairing or from aneuploid cells. Besides the one delayed P.G. with 32 chromosomes there was one other in which a hyperploid number was present but the exact number could not be counted. These two P.G's. could arise either from hyperploid cells that originated prior to MI, or from restitution at AII.

7. FERTILITY AND PROGENY

The seed fertility of the pentaploids when selfed varied from zero in one plant to 37·5 per cent. in another. These figures agree with those generally quoted for pentaploid fertility (Thompson, 1934,

and Granhall, 1943). No differences were shown by the near-pentaploids; nor were there differences between bagged heads and those left uncovered.

The seeds from the pentaploid were germinated on filter paper. The somatic complement was determined when the plants were only a few days old (fig. 2). In this manner abnormalities that might be eliminated from the somatic line could be observed. There were

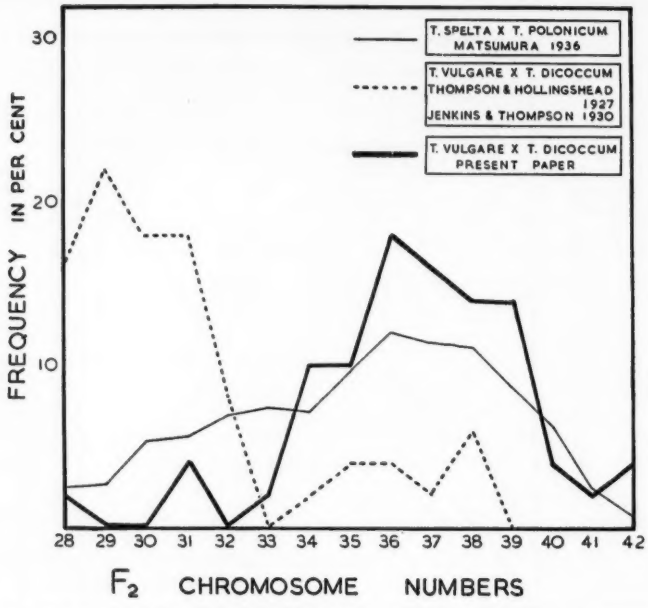

FIG. 2.—Frequency of chromosome numbers in pentaploid F_2 populations. A comparison of results for different species crosses and different localities.

16 seeds (24·2 per cent.) which did not germinate. No doubt they had unbalanced combinations in zygotes or endosperms.

In one of the root-tips with 37 chromosomes there was a dicentric chromosome and in one with 40 there was a chromosome deficient for part of an arm. No inversion bridges or trivalents were observed at meiosis so it is unlikely that the dicentric arose in this manner. Breakage (misdivision of centromeres) occurs at both TI and TII. The dicentric may have arisen from some anomalous breakage and reunion at these stages or in the resting nucleus. The short deficient chromosome could have arisen from similar breakage.

Kihara and Matsumura (1940) have advanced several reasons for the skew distribution in the F_2 of *T. polonicum* × *T. spelta*. Some of these factors: (i) competition in pollen tube growth, (ii) selective fertilisation, (iii) elimination of unbalanced gametes, either through

Plate

FIG. 3.—Photomicrographs from pentaploids. A to F 1120x, G at 1680 magnification.

A. MI, $14^{II}\ 7^{I}$.
B. MI, reduced pairing, $11^{II}\ 13^{I}$.
C. AI, abnormal separation 18 : 17, no laggards.
D. P.G. mitosis, 18 chromosomes.
E. P.G. mitosis, 16 chromosomes plus telocentric at nine o'clock.
F. 2nd P.G. mitosis, 17 chromosomes.
G. Root-tip mitosis of F_2 seedling, 38 chromosomes.

A
B
C
D
E
F
G

zygotic or endospermic media were also suggested by Thompson (1934). To explain the difference between the *polonicum-spelta* and the *vulgare-dicoccum* pentaploids of Thompson and co-workers (fig. 2) the Japanese workers supposed that more univalents were lost. Counts of chromosome numbers in P.G's. do not uphold this supposition.

Chromosome counts made on mature F_2 plants would not include any of those plants which could germinate but not survive until microsporogenesis because of their unfavourable genotype. Some of the difference between the work done in Canada on *T. dicoccum* × *T. vulgare* and my results can therefore be explained. Some of the difference may be due to chance selection and some may be an influence of environment. Certainly the dissimilarity between expected results and those observed shows that a strong selective elimination is operating and its effect has been variable in the three cited experiments.

8. SUMMARY

1. The 35 chromosomes in pentaploid wheat crosses often form less than 14 bivalents. In the 34 chromosome plants only 13 bivalents were formed and in the 36 chromosome plants trivalents occur in 60 per cent. of the cells.

2. Univalents behave similarly in pentaploid and near-pentaploid plants. At AI the univalents do not all divide : some go undivided to the poles.

3. One or more micronuclei are formed from excluded chromosomes in about 90 per cent. of the dyads and in nearly all the tetrads.

4. Centromeres of univalents misdivide at TI and TII in some cells to give telocentrics which are included in the pollen grains.

5. Pollen grains with chromosome numbers from 14 to 21 are formed in proportions departing slightly from the chance distribution of univalents.

6. F_2 progeny showed 28 to 42 chromosomes. The distribution shows strong selective elimination which differs from that in other experiments.

Acknowledgments.—I gratefully acknowledge the assistance of Dr C. D. Darlington under whose guidance the work was completed and the manuscript prepared and financial assistance from the National Research Council of Canada.

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NON-SYNCHRONISED MITOSIS IN A COMMON CYTOPLASM

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Received 10.xii.52

DURING the course of some X-ray experiments with a clone of Professor Karl Sax's *Tradescantiaz paludosa* ($2n = 12$), a control preparation of the first pollen grain mitosis showed an interesting abnormality. Along with the normal pollen grains containing the single "primary" nucleus, a large number contained two "primary" haploid nuclei. These were not the generative and vegetative nuclei since such pollen had not yet undergone any mitosis.

The presence of binucleate pollen in itself is nothing very astonishing. No doubt it arose by the complete suppression of the wall formation after the second meiotic division which would thus end in two dyads with two nuclei in each, instead of the normal tetrad containing four cells with a single nucleus in each. As expected the abnormal pollen was twice the size of the normal. The sample showed a large number of the abnormal cells undergoing first pollen mitosis and these cells clearly fell under two classes.

Only one anther was involved and even in this the abnormality was manifest only in a single lobe—the other being perfectly normal. That the abnormality was present in only one half of a single anther indeed requires the assumption of somatic mutation.

Synchronised mitosis.—In some pollen grains both the primary nuclei at any time are at exactly the same developmental stage (plate, fig. 1). This is similar to the findings of Barber (1941) and Sax (1942) on the attached pollen grains of *Uvularia* and *Tradescantia*. It also agrees with my own observations on attached and binucleate pollen in *T. bracteata* induced by different degrees of suppression of the wall formation by X-rays (Haque, 1952). The two nuclei in such cases usually complete mitosis separately to form two generative and two vegetative nuclei (plate, fig. 2). Occasionally, however, each nucleus may begin as an independent unit but, as metaphase is reached, only one plate develops: all the twelve chromosomes, six from each nucleus, then make use of the same spindle. This naturally gives a single generative and vegetative nucleus, both of which are therefore diploid.

Non-synchronised mitosis.—In a considerable number of cells, however, the development of the two nuclei was not synchronised (table and figs. 3-6).

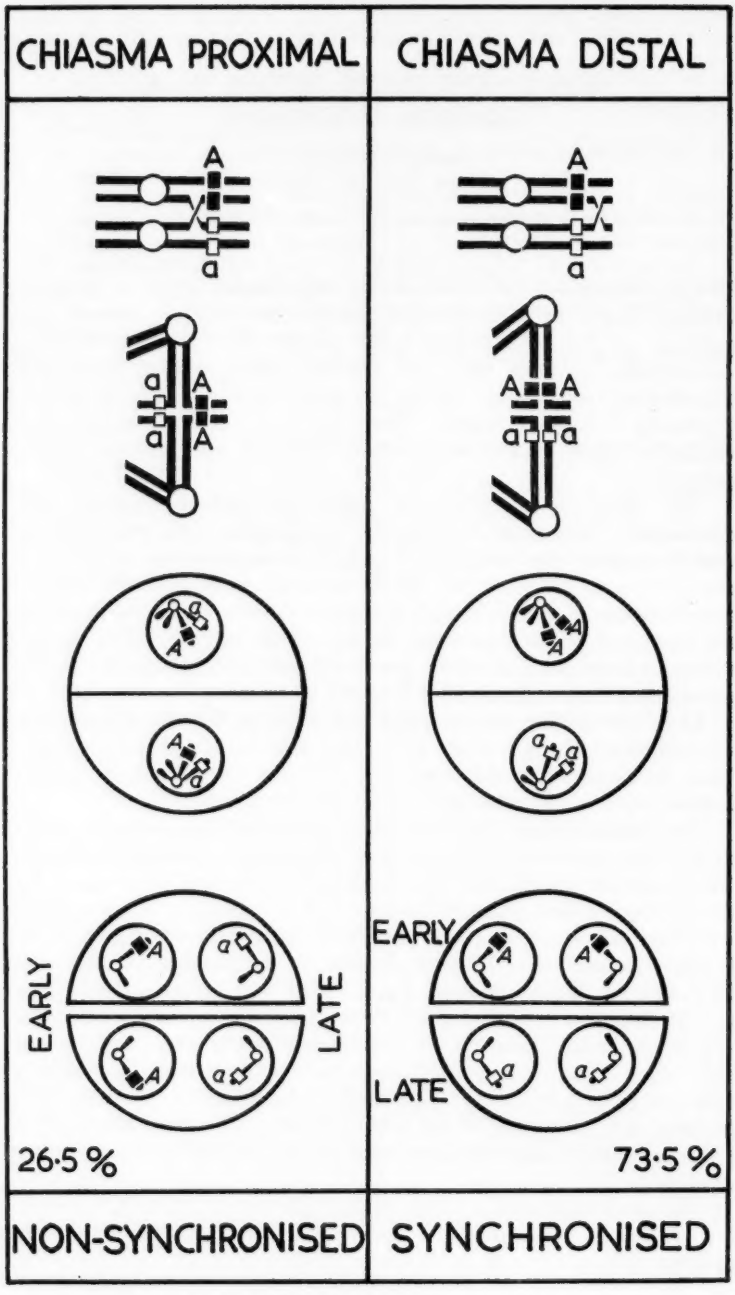


FIG. 1.—Origin of synchronised and non-synchronised mitosis in the binucleate pollen grains of *T. palusosa*.

Plate

Illustrating synchronised and non-synchronised 1st pollen mitosis in binucleate pollen of *T. paludosa*. Feulgen smear. All figures × 1750.

FIG. 1.—A binucleate pollen grain. Both the nuclei are perfectly synchronised and show late prophase of the 1st pollen grain mitosis.

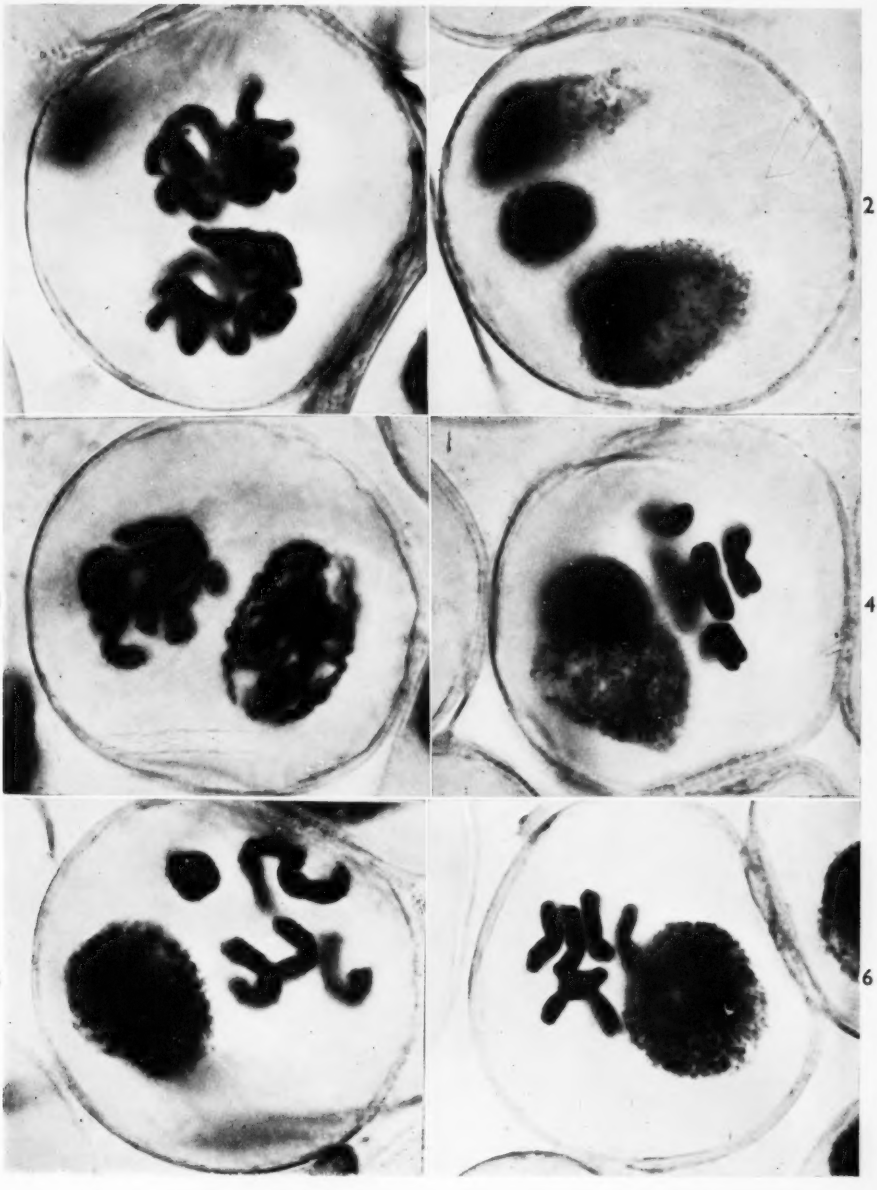
FIG. 2.—Two generative (small and densely stained) and two vegetative (large and lightly stained, one superimposed) nuclei as a result of the 1st pollen grain mitosis in a binucleate pollen grain.

FIG. 3.—Non-synchronisation. One nucleus has reached prometaphase while the accompanying one is at early prophase.

FIG. 4.—One nucleus has completed mitosis forming a generative and a vegetative nucleus while the other has as yet only reached metaphase.

FIG. 5.—One nucleus showing prometaphase while the other is still inactive.

FIG. 6.—One nucleus showing metaphase while the other is still inactive.


1
2
3
4
5
6

Barber (1941) found that sometimes, after induced meiotic abnormality, tetrads are formed where one cell contains extra chromosomes missing from its sister cell. The one with the accessory chromosomes rarely survived, the deficient one never. However, when the wall between the two was not formed, or only partly formed, both survived and the mitosis was always synchronised. This is due to cooperation between the two nuclei brought about by the flow of material from one to the other through the cytoplasm. Darlington and Mather (1949) point out that this cooperation takes place, only, if both the nuclei are equally fit or equally unfit.

TABLE

The proportion of the various classes of pollen grains in the affected anther lobe

Total no. (all classes)	Uninucleate pollen (normals)	Binucleate pollen (abnormals)		
1240	771	469		
		Total no. in mitosis	Synchronised	Non-synchronised
		87	64	23

In the present case there is no visible difference in the chromosome complement of the nuclei involved and yet some cells show synchronisation while the others do not. The dyad occurs in groups of pollen grains. The non-synchronisation is fairly evenly distributed within these groups. The synchronised and non-synchronised mitosis could be explained by assuming that this plant is heterozygous (fig. 1) for a gene (*A*) controlling the threshold for mitosis. If the gene in question is 26·5 cross-over units from the centromere, then in 26·5 per cent. of the dyads the two nuclei will have the genic constitutions *A* and *a*. In the remainder (73·5 per cent.) there being no crossing-over between the *A* locus and the centromere, there will not be any segregation at the second division. Thus the two nuclei in each dyad will be either *A* or *a*. Where pollen grains have the two nuclei *A* and *a* synchronisation will fail.

Having only half an anther for study I was not able to show that *AA* and *aa* dyad pollen grain divide at different times.

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SHANK LENGTH: SEGREGATION IN AN UNSELECTED CHARACTER IN INBRED LINES OF FOWLS

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Received 6.ii.53

THE 25-year-old flock of Brown Leghorns, maintained at the Poultry Research Centre, has been closed since 1931. Before its division into non-interbreeding lines, the descendants of its foundation birds were intermated in such a way that, with one exception, the pedigree of each line still traces back to individuals from all the five widely differing sources from which it was established. In the N line one foundation sire is missing. The general purpose line, B, has been selected, as a commercial flock might be, for both egg numbers and egg weight ; all the others have been selected for some particular trait as follows : I, intensity of production ; L, large egg ; S, small egg ; N, tendency to lay through the moulting period ; R, excessive red in the plumage ; and D, small body size. Apart from R, initiated 1939 and derived mainly from S, all have been in existence for more than 16 years.

Shank (tarsometatarsus) length was measured for the first time in September 1952. The observations were made on the pullets then coming into production, and on the yearling hens. They showed that the rank order of the lines was the same for both ages with a slight but consistent tendency for the pullet means to be greater than the yearling ones. The lines fell into two main groups, B, L, and N, with means of 95 mm. and over, and I, S, D, and R, 92 mm. and under. There was no regular association with the degree of inbreeding although it was the longest legged line of each set that was least inbred. Apart from two pairs, L and N, and D and R, the means of all the line samples, within ages, differed significantly from one another. When sire groups were considered, however, it was evident that there was a good deal of variation in line B, and that some of them were not statistically distinguishable from L and N. In contrast, line I was remarkably homogeneous ; what little variation there was in shank length was skewly distributed in that the bird frequencies piled up to a mode at the upper end of the distribution. This tendency was recognisable in all the shorter legged lines while in the others it appeared to be more normally distributed. These observations suggested that while, within the two main sets, line differences might be a matter of gene frequencies, some barrier existed beyond which the shorter legged lines could not rise ; this might be due to shank

length genes *per se* or to physiological limits set by other parts of the body.

An inherent association between skeletal variations and those of body weight is to be expected (Lerner, 1946) ; comparison of line means for the latter with those for shank length showed that the lines ranked in the same order for both, only N having slightly longer shanks than were to be expected from its position in respect of weight. This was of interest in that, until recent years, no general selection for body weight had been made. Weight differences between the lines are of long standing (Blyth, 1952) and appear to have been to some extent inherent in the genotypes necessary for the traits under selection. Thus, body weight is positively correlated with egg weight ; the small egg size arising in the S line through selection for it, and in I, through selection for the negatively correlated egg numbers, are likely to have exerted a downward pull on body weight. Again the slight increase in shank length in pullets over yearling hens may be attributable to recent pressure on body weight ; this was actually demonstrable in line B where three of the four cocks used to produce the pullets (from randomly assorted mates) belonged to the two longest shanked sire groups of the previous generation.

In two small samples of interline crosses, mean shank length was comparable with that of the longer legged parental group ; one came from lines widely divergent in egg size, and there the mean difference between reciprocal progenies was suggestive of the presence of a maternal effect.

In conclusion, it may be suggested that the different shank lengths, now characterising the various lines of the flock, result mainly, not from the random segregation of genes in the course of inbreeding, but from some unconscious directional pressure. In the early history of the lines certain bodily characteristics appear to have been associated, inevitably or otherwise, with the traits actually under selection and have become incorporated along with them.

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A CASE OF MUTUAL REDUCTION OF DOMINANCE: OBSERVED IN *MUS MUSCULUS*

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Received 30.vi.52

A DOMINANCE interaction is already known between genes at the normal/pink-eyed-dilution locus (*P*/*p*) and the black/brown locus (*B*/*b*) in *Mus musculus*. Snell (1931) reports " Pink-eyed non-agouti mice carrying brown in the heterozygous condition are distinguishable from those homozygous for black by their lighter shade." That is, *pp* is a dominance modifier of *B*.

In the stocks under my observation in this department, it now appears that *bb* is also a dominance modifier of *P*; for brown non-agouti mice heterozygous for pink-eye are distinguishable from those homozygous for non-pink-eye.

That is, there is a mutual reduction of dominance at these two loci. A table of the six distinguishable phenotypes is given below :—

	PP	*Pp*	*pp*
BB	1		2
Bb			3
bb	6	5	4

This phenomenon was first observed as a segregation of " pale " and " non-pale " among browns. Routine genetic tests were then set up which established

(i) the dominance of " pale " in *bbaa* genotypes ;
(ii) that single-factor ratios were obtained in all kinds of matings involving " pale " ;
(iii) that the " pink-eyes " produced from intercrosses of " pales," and from backcrosses of " pale " to " pink-eyes " were *pp* and not *papa* (pallid) ; and that
(iv) " pales " could be produced at will by crossing *ppbbaa* × *PPbbaa*.

In the stocks used for outcrosses and identity tests *Pp* was usually found to be " pale " ; but classification in these and the other matings was not always quite certain, and it may be possible to find stocks in which *Ppbbaa* is indistinguishable from *PPbbaa*.

The nine genotypes involving the *p* and *b* loci were made up, and several specimens of each were skinned. This work confirmed Snell's (1931) findings that *Bbpp* were distinguishable from *BBpp*.

About five skins were made of each of the genotypes 2, 3, 5 and 6 of the table. In these there was observed some phenotypic overlapping of genotypes 2 and 3, and of genotypes 5 and 6. Thus, it appears that the degree of reduction of dominance between these two loci is itself dependent on other modifying factors. It is possible, therefore, that selection in a suitably heterogeneous stock could establish complete dominance at either locus. All the genotypes in class 1 of the table appeared to have the same intensity of pigmentation ; but a larger experiment might reveal very small differences.

Two sets of skins of the nine genotypes have been made into an exhibit : one will be kept in this department and the other is in the Genetics Laboratory, Oxford. In each case, the skin selected for the exhibit, from among a set of one genotype, was that judged by eye to be median in intensity of colour for that genotype. A black-and-white photograph of the nine skins, selected in the same way from all the skins available, appears in plate 1. This gives some idea of the contrasts observable but naturally does not show them as satisfactorily as would a colour reproduction. Several attempts were made in colour, but faithful reproduction of hue as well of intensity was found to be too difficult.

From the skins it can be seen that the ratio of the intensity of colour of the genotypes 3 : 2 and 5 : 6 is about the same. That is, the action of the homozygote at each locus on the heterozygote at the other locus is roughly equivalent. It seems probable, on this evidence, that the mutant alleles at the two loci have reached about the same stage in their evolution.

On Fisher's (1931) theory of the evolution of dominance, three main factors govern the rate of evolution of a mutant at a particular locus :—

(i) the date at which the mutation first occurred at that locus. (It must be remembered that this may ante-date the differentiation of the species, often even of the genus and of higher orders) ;
(ii) the initial and subsequent viability primarily of the heterozygote and secondarily of the homozygote ; and
(iii) the subsequent mutation-rate.

It should in theory be possible to deduce any of these factors, from knowledge of the other factors and of the present interactions of mutants. As more knowledge is acquired on these points, it should become possible to make certain conclusions and to make them with increasing validity. Such knowledge will derive from various sources. Comparisons of the actions and reactions of mutants within different genera, orders and classes etc., gives a lower limit to the age of such mutants (Fisher, *ibid.*, p. 351). Initial and subsequent viabilities cannot be brought under direct observation, but fossil histories, combined with a study of the present viability of mutants on various artificially produced genetic environments, may help to give a general

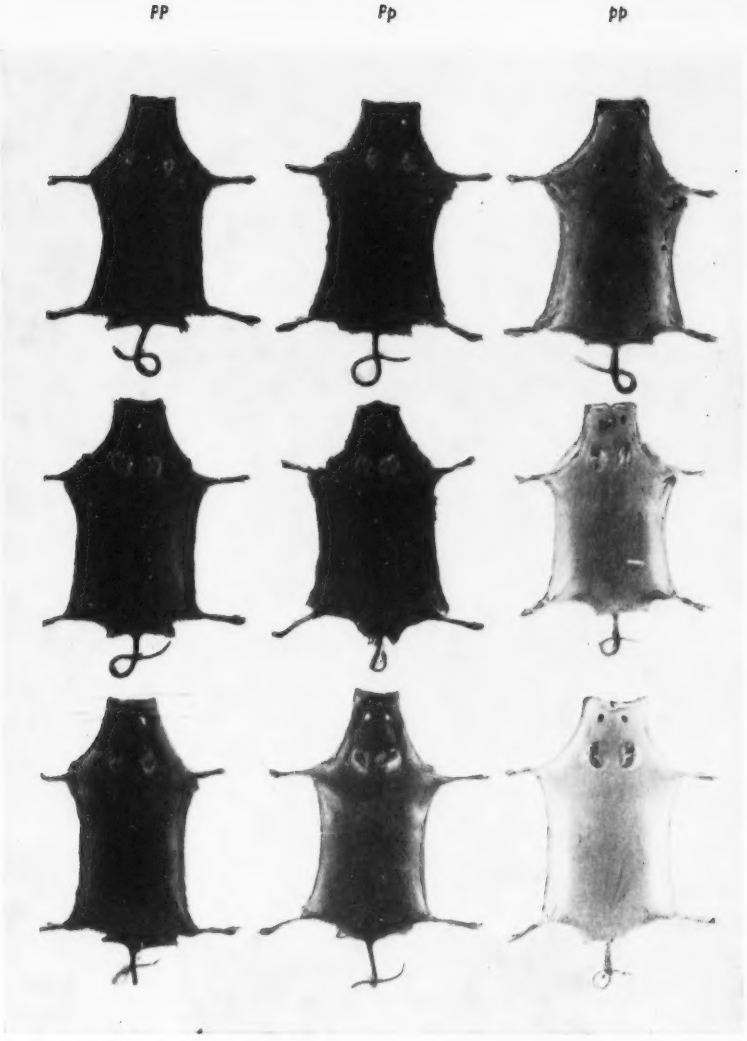

PLATE I.—Mouse skins displayed to show mutual interaction of the *p* and *b* loci. ×$\frac{1}{3}$.

idea of the initial kinds and degrees of effects of mutants which are still occurring. A history of mutation-rates is also not readily accessible. That these rates may not always have been the same has been pointed out by Fisher : " It is probable . . . that the genetic changes which have brought about evolutionary transformation of species have been accompanied by corresponding changes in the frequency and kind of deleterious mutations to which their germplasms are prone." This possibility for particular loci may also become capable of verification, with the aid of increased palæontological knowledge, and by a study of present mutation rates which do come under immediate observation.

A tentative speculation may thus be made about the evolutionary age of *p* and *b* in *Mus musculus*. Making the following assumptions, which may become verifiable, that *p* and *b* had a similar history of mutation-rate and similar initial viability, it would not be inconsistent to conclude that the two mutants first occurred at about the same period of evolution ; that is that they are about the same age.

I am indebted to Professor Sir Ronald Fisher, F.R.S., for advice and criticism of this paper, and to Miss M. A. C. Vincent for skinning some fifty specimens. I would also like to thank Messrs Oliver and Boyd for their attempts to obtain faithful colour reproductions of the skins.

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REVIEWS

MORPHOGENESIS. An Essay on Development. J. T. Bonner. 1952 London : Oxford, Univ. Press. Princeton, New Jersey : Univ. Press. Pp. i-vi+291. Price $5.00.

This is a much-needed philosophic discussion of development in plants and animals. It is delightfully written and well illustrated (although not well indexed). It contains, however, an unresolved conflict between the ideas the author began with and those he reached at the end. Dr Bonner is governed by the classical points of view of embryology. But he asserts at the beginning a wise rule that large results must be explained in terms of small causes. The classical point of view he has thought out widely and well but he has not made the fresh start that he sees the need of making with the small causes within the cell and the nucleus. He does not, for example, seem to grasp the meaning of the interlocking events in the cleavage of the *Ascaris* egg. Nor does he realise that the pollen grain and the embryo-sac are developmentally the most significant and accessible parts of the plant. No writer in this field is better qualified to make use of such knowledge and it is to be hoped that Dr Bonner will continue to develop his ideas, and on an even deeper foundation. C. D. D.

HETEROSIS. Ed. J. W. Gowen. Iowa State College Press, Ames. 1952. Pp. 552.+ix Price $5.75.

This volume comprises the thirty papers given during a five week conference on Heterosis held at the Iowa State College in the summer of 1950. As the preface points out, the State of Iowa has a direct and vested interest in heterosis, for its economy is based on hybrid corn. The conference was therefore organised with the aims of analysing the means by which this phenomenally successful development has been achieved, of considering the genetical systems on which it is based, and of exploring the possibilities of the future.

The organisers of the conference approached their task in no narrow spirit. The list of contributors does not merely call a roll of eminent maize breeders, but also includes many who are concerned with the understanding and use of heterosis in other species both domesticated and wild, and with the physiological, cytological and biochemical, as well as with the more formally genetical aspects of the problems. Some of the papers may seem to have little direct bearing on heterosis, but we can be grateful that they are included for they record work, such as that of Rhoades on peculiarities of chromosome behaviour leading to preferential segregation, which have a great interest in their own right.

Comment on each of the papers would be so demanding of space as to be out of the question, and to pick out for detailed discussion a few from the wealth that is offered to us would be invidious. The subject is approached from all points of view. The historical introduction by Zirkle, and Shull's own account of his early observations and experiments can hardly fail to be read with interest by anyone who seeks to learn the background of genetics as well as of plant breeding. Gene action is covered by several papers including an unusual and interesting discussion of seed development by Brink. Anderson and Brown, and P. Mangelsdorf consider the part played by hybridisation in the history of maize ; Irwin deals with

specificity of gene action especially in blood group determination ; and Dobzhansky discusses the adaptive significance of heterosis in populations. The notion of overdominance is, of course, taken up in a number of papers, that of Crow considering in particular its importance for the maintenance of variability in populations. Many of the authors discuss the methodology of breeding for heterosis, Sprague's paper being especially informative in the experimental evidence that it provides about the various schemes that have been proposed. A. J. Mangelsdorf sets out a series of postulates as a basis for the discussion of heterosis and other problems of plant improvement, and these can hardly fail to be of interest to geneticists and plant breeders alike, even though the author himself would hardly hope for their complete and universal acceptance.

Some of the papers may seem to many geneticists to be rather heavily statistical, but as Hull, for example, has repeatedly emphasised, only by this means can the problem of breeding for combining ability be approached with any real prospect of success. Among these statistical contributions, that of Comstock and Robinson is noteworthy for its discussion of the efficiency of experiments designed to partition the heritable variation and to measure dominance. Finally, we should note a review by the editor of the volume, J. W. Gowen, of experiments in *Drosophila*, from which a biometrical analysis of the agencies affecting heterosis was possible. His conclusion that " genes in quantitative inheritance are not stable in their effects " accords well with the evidence from other lines of research and emphasises once again the relative or conditional nature of familiar genetic concepts when one moves into the study of continuous variation.

The styles, treatments and aims of the thirty papers are as varied as their subject matter, and not all can perhaps hope to command either a wide audience or general acceptance. There is, however, something for almost every kind of geneticist, and much of the volume is essential reading for the student of continuous variation and for the practitioner of plant and animal breeding. The organisers must have been well satisfied with the success of their conference. K. MATHER.

TEXTBOOK OF GENETICS. By Wm. Hovanitz. 1953. New York. Elsevier Press, Inc Pp. i-ix+413. Price $5.95.

Genetics has come to have the most elaborate theory of all sciences. Having collected his matter the writer of a text book of genetics has therefore to find out the processes of reasoning by which the matter has developed and to illustrate these processes by a coherent arrangement of the book.

Professor Hovanitz has collected all the matter that he needs, although not perhaps remembering very well where it came from. But he trips over the reasoning and the arrangement. At the beginning he attempts no clarification or definition of ideas. He distinguishes on page 1 emphatically in italics between " *breeding* " and " *experiment* ". But is breeding not an experiment ? On the same page he offers us the " germplasm " without any explanation, as though we all knew what that was. But does the author himself know ? Introducing his chapter 16, Hovanitz reveals his own mode of reasoning. " That there must be a relation between the gene and the character follows from observation that the substitution of one allele for another results in the substitution of one character for another." But surely it does not " follow from the observation " ; it merely paraphrases

the inference. It is indeed a tautology. Similarly to Hovanitz " A second cause of the failure of double cross-over classes to appear is known as *interference* " (p. 155). In this way the reader is invited to skip one of the most interesting hypotheses in the development of genetics.

Professor Hovanitz's failure to distinguish between reasoning, repetition and convention is fairly consistent. It is, we may suppose, a symptom of a general failure to know the difference between descriptive and theoretical science. In the circumstances it is unfortunate that he should choose Genetics for his subject. In other sciences a comparable effort might have yielded a less dislocated result. All that would have been noticed would have been the solecisms and malapropisms, the bibliographical errors and inconsistencies, and the mere misspellings and misprints which, beginning with the first word, splash the work with unexpected colours. The reviewer does not complain of these irregularities but rather feels relieved that the author did not take too much pains with his book.

C. D. D.

GENETICAL SOCIETY OF GREAT BRITAIN

ABSTRACTS of Papers read at the HUNDRED AND ELEVENTH MEETING of the Society, held on 16th APRIL 1953, at the GALTON LABORATORY, University College, London, W.C. 1

SOME PROBLEMS OF CHAETA VARIATION IN *DROSOPHILA*

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The abdominal sternites on three segments in males and four in females of *Drosophila melanogaster* carry roughly equal numbers of chaetæ, so that sternite chaeta number may be considered as a metameric quantitative character, repeated twice in males and three times in females. One might expect to find high correlations between the different segments due to both genetic and environmental variations, and progeny tests show that the genetic correlations are close to unity —*i.e.* gene differences alter the general level of chaeta production on all sternites, and cause little or no local variations.

But counts on inbred lines indicate that there is virtually no non-genetic correlation between even adjacent segments. Chaeta number is also changed remarkably little by moderate changes in environment which have marked effects on other quantitative characters, so that it appears to be little correlated with the normal environmental variables. Finally, the variance of chaeta number was no greater in inbred lines than in crosses between them, in contrast to measures of body-size, whose variance is very much less in the crosses than in the parent inbred lines.

These facts lead to the hypothesis that the non-genetic variance of chaeta number is mainly, in our culture conditions, a " chance " variability of extremely localised origin, and not a true environmental variability. Some possible causes of this variability will be discussed.

MASS SELECTION FOR LARGE AND SMALL SIZE IN *DROSOPHILA*

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Mass selection for large and small body size has been carried out in a number of unrelated wild stocks of *Drosophila melanogaster* ; progeny tests showed that these stocks contain a great deal of genetic variability. The response to selection was immediate and approximately linear for a number of generations. Parallel selections in the different stocks lead to very similar results. There was asymmetry in the response to selection in the two directions, since the strains selected for small size changed size more rapidly and also made a greater total progress than the strains selected for large size.

All the large strains retained considerable genetic variability after they had ceased to respond to selection ; this was demonstrated by reversing and relaxing selection. The small strains are being tested in the same way.

The effect on size of inter-crossing the different related and unrelated strains has been studied, and selection has been carried out on crosses between large and between small strains.

IMPERFORATE ANUS IN MICE: A STUDY IN GENE-INTERACTION

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The recessive mutant "vestigial tail" (symbol *vt*) was tested in combination with three other mutants affecting the tail—"undulated" (*un*), "Short-Danforth" (*Sd*) and "Brachyury" (*T*).

The combinations *vt un* and *vt Sd* were viable, fertile in both sexes, and free from any gross morphological anomalies attributable to gene-interaction. On the other hand, mice of genotype $vt/vt/T/+$, although born alive in both sexes, were without exception tailless and in every case the anus was imperforate.

In male imperforates the urethra opened into the colon, which ended blind. In females the alimentary, reproductive and urinary tracts were confluent and opened to the exterior by a common cloacal aperture. The males all died at the end of the first week of life, but some imperforate females survived to maturity.

These observations, when combined with the results of similar crosses performed by Prof. L. C. Dunn (unpublished), showed that the manifestation of the imperforate syndrome (though not of the tailless condition) in mice of the doubly mutant genotype was extremely sensitive to genetic modifiers differentiating Dunn's stocks from mine.

Thus it appears that the interaction of two mutant genes, given a certain genetic background, can with regularity produce a character normally judged to be of high importance in taxonomy—the presence of a cloaca in the Mammalia.

ESTIMATING FREQUENCY OF VISIBLE RECESSIVE MUTATIONS DETECTED IN INBRED LINES

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Computational methods are described for estimating the frequency of spontaneous or induced visible recessive mutations, detected in inbred lines maintained by a single sib-mating in each generation, with perhaps an occasional intercalated backcross. These involve the repeated use of square arrays, or matrices, of multipliers whose algebraic properties lead to simple rules for assessing the efficiency of any given experimental procedure. Due allowance can be made for varying sizes of sibship, and for incomplete penetrance and partial lethality. By an extension of the methods described, it would be possible to use any inbreeding programme as a source of information on spontaneous mutation rates.

THE CYTOLOGY OF INTERSPECIFIC HYBRIDS BETWEEN THE NEWTS *TRITURUS CRISTATUS* AND *T. MARMORATUS*

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F_1 male hybrids show a great reduction in chiasma frequency as compared with the parent species. They are completely sterile: F_1 female hybrids are fertile. Backcross male hybrids show a wide range of chiasma frequencies, some being as low as F_1 male, some nearly as high as the male species parent. Two out of seven diploid backcross male hybrids form multivalents at meiosis: a number of translocations appear to differentiate the parent species. The meioses of three triploid backcross male hybrids have also been analysed.

LOCAL POPULATION DIFFERENTIATION IN *AGROSTIS TENUIS*

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In order to find out what differentiation there was between natural populations of *Agrostis tenuis* (Bent grass) occurring in a small geographical region round Aberystwyth, samples were taken and grown together in an experimental garden for several years. A considerable amount of differentiation was found, the population of any one area being apparently adapted to the environment of that area.

This was true even when populations sampled were only 100 yards apart, provided that the environments of the populations were markedly different. This difference seems rather little to isolate properly two populations from one another, especially since *A. tenuis* is wind pollinated. But recent work by other authors suggests that the amount of cross pollination that could be expected under such conditions is as low as 5 per cent. This gene flow would easily be overcome by natural selection.

PHENOTYPIC STABILITY AND ALTERNATIVE GENE PATHWAYS

D. LEWIS
John Innes Horticultural Institution, Hertford

The maintenance of a phenotype within fairly narrow limits (*phenotypic stability*) under different environments is a factor of considerable importance in evolution and also in practical breeding. An attempt has been made to analyse the genetic factors affecting phenotypic stability both within populations and within individuals of *Lycopersicon esculentum*. A quantitative character was chosen which was markedly affected by an environment which could easily be controlled. The character was the number of flowers per inflorescence, and the environment was temperature during early growth. Means, variances, and *stability factors* have been analysed for two inbred parents, F_1, F_2, 20 F_3 and backcross generations which had been raised in two different temperatures. The results are consistent with an interpretation based upon a positive correlation between phenotypic stability and the number of alternative gene pathways depending upon heterozygosis, dominance and epistasy.

INCOMPARIBILITY IN THE COMPOSITAE

LESLIE K. CROWE
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Incompatible species of Angiosperms have been classified into two systems.

(i) With one locus and multiple alleles. The action of genes in the style is individual. The control of the pollen is gametophytic. Homomorphic plants.

(ii) With one or two loci and two alleles per locus. The control of the pollen is sporophytic with alleles showing dominance in both pollen and style. Heteromorphic plants.

Recent studies in the United States on two Composite plants revealed that the breeding systems do not conform to either of the above types. The facts were explained on a third system which combines features of the other two. Another example in *Cosmos bipinnatus* will be described which is interpreted in a similar way.

(iii) One locus with multiple alleles. The control of the pollen is sporophytic, the alleles showing dominance or individual action in both pollen and style according to the combination in which they are found.

INTERCHANGE BY MISDIVISION IN *TRITICUM*

J. W. MORRISON
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An interchanged chromosome and also a telocentric chromosome arose in the progeny of a monosomic of hexaploid wheat. The telocentric was recognised by its morphology and the interchange was inferred from quadrivalent and trivalent chromosome associations which do not occur in normal monosomic plants. In the parent one or three univalent chromosomes undergo misdivision at meiosis to form telocentrics. Since the interchange affects one of the same chromosomes as the misdivision which gave the telocentric, the interchange is assumed to result from the fusion of two telocentrics from non-homologous chromosomes.

ABSTRACTS of Papers read at the HUNDRED AND TWELFTH MEETING of the Society, held on 9th and 10th JULY 1953, in the BOTANY DEPARTMENT, University of Manchester

ECOLOGICAL AND GENETICAL STUDIES IN *DROSOPHILA MELANOGASTER* MEIG.

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An apparatus has been devised in which the white eye mutant of *Drosophila melanogaster* Meig. is at an advantage compared with the wild type. Selection effects for migratory reaction were observed under conditions in which flies experienced difficulty in finding food. The migratory behaviour of the Cambridge white-eyed type was compared with various wild (red eyed) strains. These results will be discussed.

THE CYTOLOGY OF PRIMARY, TRANSPLANTED AND ASCITES TUMOURS

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A detailed cytological analysis made on spontaneous primary and induced tumours as well as transplanted series of carcinomas and sarcomas in several strains of mice, rats and two species of hamsters has given interesting information about he structural charactetistics and distinctive scatter and modality of chromsomal distribution in the cell population. While the cells of the primary tumour are almost entirely diploid, that of the transplanted generations whether solid or ascites conform to three distinct types : the predominantly diploid, the markedly tetraploid and finally intermediate heteroploid types exhibiting a shift towards tetraploidy. Since the primary tumours in all cases investigated are diploid the cells of the aneuploid series would seem to be drifting to a new chromosomal equilibrium independently of the original chromosomal make-up of primary origin. The aneuploid series are also characterised by the presence of supernumerary fragment chromosomes and one or more metacentric chromosomes. The anomalies and errors of chromosome mechanics are also discussed.

A NEW HYPOTHESIS FOR "CHROMATID" ABERRATIONS

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A new hypothesis has been developed, primarily to account for visible chromosome changes induced by radiomimetic chemical compounds in *Vicia* root tip cells. All changes previously interpreted as chromatid or isochromatid breaks, or as resulting from reunion between these breaks, are assumed instead to arise from the induction of chromatid exchanges. It is supposed that such exchanges may be induced to occur only at points of chromosome association, and that the process of their formation is similar to that of meiotic chiasmata. Interpreted in this way the observations do not provide any direct evidence of breakage. It is proposed that this hypothesis should be extended to include X-ray aberrations of the same type.

THE ORIGIN OF *ROSA WILSONI* BORR.

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Dept. of Botany, Manchester University

Rosa wilsoni Borr. is a hexaploid hybrid ($2n = 42$) native to this country, originally described from a locality on the Menai Straits near Bangor. It probably derives 28 chromosomes from *R. canina* and 14 from *R. spinosissima*.

THE GENETIC EFFECT OF ARTIFICIAL INSEMINATION ON MILK PRODUCTION

ALAN ROBERTSON and J. M. RENDEL
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An analysis has been made of some 1500 heifer lactations of cows got by artificial insemination between 1942 and 1948. A comparison with other heifers milking in the same herds at the same time showed a difference in favour of the A.I. animals of 1 gallon in average lactation yield and 0·06 per cent. in fat content. The superiority of the A.I. animals was apparently the same in herds at all levels of production. The genetic implications of these findings will be discussed.

THE EFFECT OF DIET ON VARIATION IN A PURE LINE OF MICE

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The pure line C 57 Black shows extensive skeletal polymorphism mainly due to intangible non-genetic factors, though maternal age, litter-size, etc. are sometime important. In experiments designed to find out more about the physiological basis of this variation it has been discovered that the change from a normal rat-cake diet to one of oats and hay leads to marked alterations in the frequencies of a number of variations. One anomaly, in which the spinous process of the second thoracic vertebra is split into two halves, is absent from the controls but present in 16·4 per cent. of those on oats. It is known that this particular anomaly is also common in an American substrain of C 57 Black, fed on a normal diet. Thus a simple nutritional change can alter the manifestation of an anomaly in the same way as a change in the genetic background ; in other words, can produce a phenocopy.

PHAGE TRANSDUCTION OF FLAGELLAR CHARACTERS IN *SALMONELLA*

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Some *Salmonella* phages show transducing activity, that is they can carry over genetic material from their previous host to a small fraction of the cells of a new host which absorb the phage, but are not killed by it. Non-motile strains treated with bacteria-free lysates of other strains produce stable motile forms detected as spreading swarms in semi-solid agar. Non-flagellated mutant strains produce swarms with the flagellar antigen(s) characteristic of their own species, and otherwise untypeable non-flagellated strains can be assigned to species by the flagellar antigens of their induced swarms.

The production of swarms by non-flagellated strains when treated with lysates of other non-flagellated strains demonstrates the existence of at least six genetic factors mutation of any of which may cause absence of flagella, thus masking other genes which regulate flagellar antigens.

In some exceptional cases only, two distinct flagellar characters may be simultaneously transduced, *e.g.* flagellation and a flagellar antigen ; this perhaps indicates that the pairs of genes concerned are linked.

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